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The Hippo Pathway and YAP/TAZ-TEAD Protein-Protein Interaction as Targets for Regenerative Medicine and Cancer Treatment

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## The Hippo Pathway and YAP/TAZ-TEAD Protein-Protein Interaction as Targets for Regenerative Medicine and Cancer Treatment

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5 **The Hippo Pathway and YAP/TAZ-TEAD Protein–Protein Interaction as Targets for**  
6 **Regenerative Medicine and Cancer Treatment**  
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5 **Abstract:** The Hippo pathway is an important organ size control signaling cascade, acting as the  
6 major regulatory mechanism of cell-contact inhibition. Yes Associated Protein (YAP) and  
7 Transcriptional co-Activator with PDZ-binding mot (TAZ) are its targets and terminal effectors:  
8 inhibition of the pathway promotes YAP/TAZ translocation to the nucleus, where they interact with  
9 Transcriptional Enhancer Associate Domain (TEAD) transcription factors family and co-activate  
10 the expression of target genes, promoting cell proliferation. Defects in the pathway can result in  
11 overgrowth phenotypes due to the modulation of tissue-specific stem-cell proliferation and  
12 apoptosis, and members of the pathway are directly involved in cancer development. The  
13 pharmacological regulation of the pathway might be beneficial in cancer prevention and treatment  
14 and useful for regenerative medicine applications; however, currently, there are very few  
15 compounds able to selectively modulate the pathway. In this review, we present an overview of the  
16 Hippo pathway, the sequence and structural analysis of YAP/TAZ, the known pharmacological  
17 modulators of the Hippo pathway and the design of new modulators to target the YAP/TAZ-TEAD  
18 interaction.  
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31 **Keywords:** Hippo pathway; stem cells; TAZ; YAP, TEADs; Verteporfin.  
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## INTRODUCTION

Coordination of cell proliferation and death is essential not only to attain an appropriate organ size during development but also to maintain tissue homeostasis during postnatal life. The mechanism by which multicellular organisms orchestrate the growth of their individual cells and the size of their organs is a longstanding puzzle in developmental biology that still remains to be resolved. Signaling pathways converting extra- and intra-cellular events into gene transcription are pivotal in cell number regulation.

Recently, a newly discovered and evolutionarily and functionally conserved signaling kinase-cascade, the “Hippo pathway”, has been shown to play a critical role in controlling organ size by regulating both cell proliferation and apoptosis [1-5]. Initially, this pathway was discovered in *Drosophila melanogaster* by mosaic genetic screens, which proved to be a powerful tool in the elucidation of this molecular signaling. Each member of this pathway in *Drosophila* has a correspondent counterpart in mammals sharing a high analogy- and homology-level with it. Several genetic and biochemical studies gradually demonstrated that the *Drosophila* mutants for any of the signaling pathway components exhibit an overgrowth phenotype (as a *hippopotamus*), leading to the current model in which the Hippo pathway is the major regulatory mechanism of cell-contact inhibition [2, 6]. The terminal effector-component of the Hippo pathway is a transcription co-activator, named Yorkie (Yki) in *Drosophila*. In mammals, Yes-Associated Protein (YAP) and its paralogue Transcriptional co-Activator with PDZ-binding motif (TAZ) have been identified as terminal effectors of the pathway [7]. The final effect of the Hippo pathway on the Yki/YAP/TAZ proteins involves phosphorylation on specific Serine residues, to confine them in the cytoplasm for subsequent degradation. Consequently, Yki/YAP/TAZ cannot translocate to the nucleus, where they would bind to other proteins (including TEADs in mammals) and act as transcription co-activators, triggering the expression of cell proliferation-promoting genes [1, 2, 8].

Hippo signaling alterations are increasingly being associated with cancer development [4, 9-11]. It was observed that a significant percentage of patients affected by certain cancers, such as cancer of the liver, breast or pharynx, harbor causative amplification or over-expression of the YAP gene; therefore, the use of YAP-inhibiting drugs could be tailored to the selected patients for optimal effects [12]. These clinical observations suggest that the pathway, particularly the YAP-TEAD complex, can be targeted to inhibit cancer or modulate proliferation. Because the YAP-TEAD complex corresponds to the final step of YAP activity, its targeted inhibition should diminish the potential side effects expected from targeting the upstream proteins of the pathway, which are more interconnected with other signaling networks. Therefore, the protein-protein interactions between

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3 YAP and TEAD emerge as the best candidate target for modulating the Hippo pathway with small  
4 molecules.  
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10 Currently, there are no known compounds that can interfere in a consistent manner with the YAP–  
11 TEAD interaction. However, the YAP–TEAD complex structure has been resolved by X-ray  
12 crystallography, and mutagenesis studies have identified the amino acid residues crucial for forming  
13 the functional complex [13]. This structural information could allow the design of new compounds  
14 directed to the target protein. Consequently, modulating YAP activity could possibly help regulate  
15 the self-renewing, proliferative potential and the fate of stem cells and could hopefully direct stem  
16 cell differentiation towards a specific cell-lineage. From this perspective, the following points  
17 should be considered: (i) the Hippo pathway is involved in cross-talk with several growth regulatory  
18 signaling pathways; (ii) not all tissue-specific progenitors are regulated by this pathway and (iii)  
19 different sets of downstream target genes are regulated in a tissue-specific manner. Hence, the  
20 number of cross-linking signals could be very high, triggering a significant redundant and  
21 compensatory mechanism. Despite these limitations, a tissue-specific modulation of YAP activity  
22 could represent a powerful tool for future regenerative medicine applications. This review provides  
23 an overview of the Hippo signaling cascade, explaining the role of this pathway in differentiated  
24 cells and stem cells throughout organ development. An emphasis will be placed on the importance  
25 of the YAP–TEAD complex as a drug-target and on the possibility of using a medicinal chemistry  
26 approach to develop compounds that can interfere with stem cell fate, through this target, for future  
27 regenerative medicine applications.  
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### 45 **Biology of the Hippo pathway**

#### 46 47 **THE HIPPO SIGNALING PATHWAY AND YAP/TAZ REGULATION**

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50 The Hippo signaling pathway was initially described in *Drosophila*, where it gradually emerged as  
51 a major regulatory mechanism of contact-inhibition during the growth of the cell [1-5]. Numerous  
52 genetic and biochemical studies in *Drosophila* have led to a extensive characterization of the  
53 pathway: it consists of a series of serine/threonine phosphorylation events that lead to the inhibition  
54 of cell proliferation and the promotion of apoptosis via inhibition of the transcriptional co-activator  
55 Yki. The pathway is regulated by a variety of upstream modulators, including transmembrane  
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3 receptors belonging to the cadherin family (Ft, Ds) and components of tight-junctions (aPKC, Par3,  
4 Par6), adherens junctions (a-Catenin) or apical-basal polarity protein complexes (Crb, PatJ, Std,  
5 Lgl, Scrib, Dlg; see Tab. 1) [1, 2, 9]. Upon initiation of the pathway, a complex comprising Ex, Mer  
6 and Kibra proteins is formed, which in turn activates the core Hippo pathway components (Hpo,  
7 and Kibra proteins is formed, which in turn activates the core Hippo pathway components (Hpo,  
8 Sav, Mats and Wts). Consequently, the transcriptional co-activator Yki is phosphorylated on  
9 multiple sites, thereby creating a 14-3-3 binding site and resulting in cytoplasmic retention and  
10 inactivation of Yki. When the Hippo pathway is inactive, Yki bypasses the phosphorylation by Wts  
11 and enters the nucleus, where it binds and activates a transcription factor (Sd). This induces several  
12 downstream target genes, including promoters of cell-growth (Myc and Ban), promoters of cell-  
13 cycle progression (E2F1, cyclin A, B and E), inhibitors of apoptosis (Diap1) and components of  
14 other important signaling pathways [2, 8, 9].  
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24 The Hippo pathway is highly conserved in mammals. Ft, Ds, Ex, Mer and Kibra homologues (Ft1-  
25 4, Dchs1-2, FRMD6/Ex1, NF2 and WWC1/WWC2; see Tab. 1) do exist; however, their functional  
26 significance in regulating the pathway is just beginning to be delineated, and further investigation is  
27 needed to characterize the steps that initiate the pathway in mammalian cells [9, 14]. The core  
28 components and downstream effectors of the *Drosophila* pathway are highly conserved: Mst1/2  
29 (Hpo homologues), Sav1 (Sav homologue), Lats1/2 (Wts homologues), MOBKL1A and  
30 MOBKL1B (collectively referred to as Mob1; homologues of Mats), and YAP and its paralogue  
31 TAZ (also called WWTR1; homologues of Yki) (Tab. 1) [4, 15]. Relationships among Hpo, Sav,  
32 Wts, and Mats are also conserved among mammalian Mst1/2, Sav1, Lats1/2, and Mob1 (Fig. 1).  
33 NF2, FRMD6 and WWC1/2 interact and promote activation of the Mst1/2-Sav1 complex by a  
34 mechanism that is not fully characterized [9]. As with the *Drosophila* Sav and Hpo proteins, the  
35 mammalian Sav1 protein interacts with and activates the serine-threonine kinases Mst1/2 through  
36 the SARAH domains present in both; however, the underlying mechanism is still unclear. The auto-  
37 activation of Mst1 and 2, which occurs by phosphorylation on threonine residues within their  
38 activation domain, triggers the phosphorylation and consequent activation of their direct substrates,  
39 Lats1/2 [16, 17]. The latter forms a complex with Mob1, which is phosphorylated by Mst1/2,  
40 resulting in an enhanced Lats1/2–Mob1 interaction. The activated Lats1/2–Mob1 complex in turn  
41 phosphorylates YAP (on Ser127) / TAZ (on Ser89), preventing their nuclear translocation via  
42 binding to cytoplasmic 14-3-3 proteins and thus playing the most critical role in regulating  
43 YAP/TAZ nuclear-cytoplasmic localization. Mutation of Ser127 in YAP and subsequent disruption  
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3 of the 14-3-3 binding site activates YAP, further confirming the inhibitory role of the  
4 phosphorylation on this specific residue [18, 19].  
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7 Although several upstream modulators of the pathway identified in *Drosophila* have structural and  
8 functional homologues in mammals, certain relevant differences have been highlighted. In contrast  
9 to dRASSF function in *Drosophila*, most mammalian RASSF homologues are scaffold proteins  
10 activating Mst1/2: RASSF1A, which has the highest homology with the fly protein dRASSF,  
11 activates the Mst1/2 kinases by preventing their dephosphorylation by PP2A [9, 20, 21]. Another  
12 Ras effector family member, RASSF6, binds to Mst2 and antagonizes Hippo signaling [22]. As in  
13 *Drosophila*, Ajuba (homologue of *Drosophila* Jub) physically interacts with Lats1/2 and Sav1,  
14 leading to the inhibition of the Hippo signaling activity [23]. The presence of conserved *Drosophila*  
15 orthologues in the mammalian Hippo pathway may serve as a mechanism of redundancy that  
16 protects the organism against cancer-causing mutations.  
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29 At the nuclear level, YAP/TAZ associate with TEAD1-4 (homologues of *Drosophila* Sd) and  
30 stimulate the transcription of genes involved in the control of cell-proliferation, differentiation and  
31 development, such as Myc [24, 25], Gli2 [26], CTGF and Cyr61 [26-28]. The TEAD family  
32 transcription factors are the main YAP/TAZ partners in the regulation of gene expression.  
33 Knockdown of TEADs or disruption of the YAP–TEAD interaction abolishes YAP-dependent gene  
34 transcription and substantially diminishes YAP-induced cell proliferation and oncogenic  
35 transformation [4, 10, 29]. A mutation of TEAD1 Tyr421, which forms a hydrogen bond with YAP,  
36 results in loss of interaction with YAP and leads to the human genetic disease Sveinsson's  
37 chorioretinal atrophy [30]. Precise regulation of the YAP–TEAD interaction is therefore important  
38 in maintaining normal physiology. Despite a major role for TEADs in YAP/TAZ function, other  
39 transcription factors containing PPXY-motifs are known to interact with the WW-domains of  
40 YAP/TAZ, including Smad1, Smad2/3, RUNX, ErbB4 and p73 for YAP [4, 31], and RUNX,  
41 PPAR $\gamma$ , Pax3, TBX5 and TTF-1 for TAZ [8, 32-35]. The interaction of YAP with Smad1 is  
42 important for maintaining the pluripotency of mouse embryonic stem cells (mESCs), mediated by  
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3 the activation of the Bone Morphogenetic Protein (BMP) transduction pathway (Fig. 2A).  
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5 Considering that BMP plays a role in mESC self-renewal and differentiation and that both BMP-  
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7 and Hippo-pathways have the ability to control organ size, the regulated interaction of Smad1 and  
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9 YAP could possibly mediate the cross-talk between the two signaling cascades [36]. Additionally,  
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11 YAP and TAZ bind Smad2/3. In human embryonic stem cells (hESCs), Smad proteins are  
12  
13 transcriptional modulators through which TGF- $\beta$  family members regulate many developmental  
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15 events (Fig. 2B). Particularly, the YAP–Smad2/3 interaction is believed to dictate the nuclear  
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17 accumulation of Smad2/3 for subsequent transcription activation. In this manner, YAP regulates  
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19 Smad nuclear localization and coupling to the transcriptional machinery [37]. YAP also interacts  
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21 with p73, a p53 family pro-apoptotic transcription factor, to induce the expression of genes such as  
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23 Bax, Puma and PML [38]. Another YAP interaction partner is RUNX2, which stimulates the  
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25 osteoblastic differentiation of mesenchymal stem cells (MSCs), to promote chondrocyte  
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27 hypertrophy and to contribute to endothelial cell migration and vascular invasion in bone  
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29 development. YAP interacts with the full-length RUNX2 protein, as well as RUNX2-responsive  
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31 promoter regions; however, the effects of YAP on the expression of the RUNX2 target genes appear  
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33 to depend on the promoter, namely on the cohort of other DNA-binding proteins and co-factors  
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35 brought to the gene by specific DNA sequences and protein–protein interactions [39, 40]. ErbB4 is  
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37 a tyrosine-kinase receptor protein which is proteolytically processed by membrane proteases in  
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39 response to the ligand, resulting in the translocation of its cytoplasmic COOH-terminal fragment  
40  
41 (CTF) to the cell nucleus. YAP may associate with the cytoplasmic portion of the ErbB4 receptor  
42  
43 and co-activate transcription mediated by CTF. Thus, the CTF of ErbB4 produced by  $\gamma$ -secretase  
44  
45 cleavage, translocates, along with YAP, to the nucleus upon ligand stimulation, and YAP may act  
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47 not only as a transcriptional co-activator for the CTF but also as a carrier protein for translocating  
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49 the CTF from the membrane to the nucleus [41].  
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3 Phosphorylation of Yap at Ser127 promotes its binding to the 14-3-3 proteins and its subsequent  
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5 cytoplasmic sequestration and inactivation; however, YAP phosphorylation can also induce its  
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7 degradation: Lats1/2 phosphorylates YAP at Ser381, which primes YAP for subsequent  
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9 phosphorylation by another kinase, possibly Casein Kinase 1 (CK1 $\delta/\epsilon$ ). This might activate a  
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11 phosphorylation-dependent degradation motif, termed phosphodegron. Subsequently, the E3-  
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13 ubiquitin ligase SCF $\beta$ -TRCP is recruited to YAP, leading to its polyubiquitination and degradation.  
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15 YAP/TAZ can also be inhibited through protein-protein interactions, in a phosphorylation-  
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17 independent manner, resulting in their cytoplasmic sequestration [18]. Recently, YAP/TAZ and  
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19 Angiomotin family proteins (AMOT) were shown to interact, resulting in YAP/TAZ inhibition  
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21 through various mechanisms. AMOT was first identified as an “Angiostatin binding protein”  
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23 promoting endothelial cell migration and angiogenesis, and due to its angiogenic function, it has  
24  
25 been implicated in tumor growth. However, the angiostatin-responsive migration-promoting  
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27 functions are observed in the AMOT p80 splicing variant that does not bind YAP, but not in the  
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29 YAP-binding p130 variant. The AMOT family has three paralogues in humans and mice (AMOT,  
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31 AMOTL1 and AMOTL2) and two isoforms generated by alternative splicing: the p80 isoform lacks  
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33 400 amino acids at the N-terminal present in the p130 isoform. YAP interacts with AMOT p130  
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35 alone because the p130 unique N-terminal region contains two PPXY motifs, which serve as  
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37 binding partners for the YAP WW-domains. Mutation of the first PPXY motif, which is conserved  
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39 in all three AMOT family members, significantly decreases the interaction with YAP. Mutation of  
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41 the second PPXY motif, which is not conserved in AMOTL2, has little effect. The combined  
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43 mutation of both PPXY motifs shows an effect similar to the mutation of the first PPXY motif.  
44  
45 Therefore, the WW-domains of YAP and the first PPXY motif of AMOT play major roles in the  
46  
47 YAP-AMOT interaction [42]. AMOT can inhibit YAP/TAZ by various mechanisms. Through  
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49 physical interaction, AMOT recruits YAP/TAZ to various compartments such as tight-junctions and  
50  
51 actin cytoskeleton, depending on the cellular location of YAP/TAZ: AMOT binds to Pals and PatJ, thus  
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53 forming the tight junction-related Crumbs cell-polarity complex, which sequesters the  
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3 transcriptional co-activators YAP/TAZ at sites of cell–cell adhesion, preventing their nuclear  
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5 localization [43-45]. In addition, AMOT is present in protein complexes along with ZO-1/2 and  
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7 PTPN14, which mediate the cytoplasmic retention of YAP/TAZ, independent of their  
8  
9 phosphorylation status. ZO-2 was reported to consistently inhibit TAZ-mediated transactivation and  
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11 YAP2-dependent induction of proliferation [46, 47]. AMOT family proteins additionally inhibit  
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13 YAP/TAZ by promoting their inhibitory phosphorylation; however, it is unclear whether induction  
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15 of YAP phosphorylation indirectly depends on the modulation of its subcellular localization. Other  
16  
17 known modulators of YAP/TAZ belong to adherens junctions:  $\alpha$ -Catenin, an adherens junction  
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19 component and a tumor suppressor, promotes YAP cytoplasmic localization and inhibits YAP  
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21 activity by interacting with and stabilizing the YAP1/14-3-3 complex [48, 49]. Dlg and Scrib  
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23 proteins are associated with baso-lateral polarity complexes and increase YAP/TAZ  
24  
25 phosphorylation; however, the molecular mechanisms underlying this process in mammalian cells  
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27 are still poorly understood [9, 50].  
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### 35 **HIPPO SIGNALING PATHWAY AND STEM CELLS**

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38 Over the last decade there has been growing evidence that the Hippo pathway can affect tissue size  
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40 by directly regulating stem cell (SCs) proliferation and maintenance. Numerous studies have  
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42 investigated Hippo signaling in various stem cell populations and have revealed its crucial role in  
43  
44 stem cell biology: in general, YAP overexpression or inactivation of any Hippo signaling pathway  
45  
46 component promotes proliferation and prevents differentiation of different tissue-specific SCs.  
47  
48 However, not all tissue-specific progenitors are regulated by this pathway, and its manipulation  
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50 leads to different effects depending on the specific cell-type considered. Consequently, the  
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52 manipulation of Hippo signaling regulation may represent a possibility to influence stem cell  
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54 commitment and differentiation towards a specific and unique cell-lineage. Thus, the manipulation  
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3 of the Hippo regulation mechanism may represent a useful tool in clinical applications and  
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5 regenerative medicine.  
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### 10 11 *EMBRYONIC STEM CELLS (ESCs)* 12

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14 Embryonic stem cells are isolated from the Inner Cell Mass (ICM) of blastocysts. They are  
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16 pluripotent stem cells because they have the ability to give rise to all cells and embryo tissues,  
17  
18 except placental extra-embryo tissue. Therefore, they represent the source of all tissues comprising  
19  
20 the developing embryo, the fetus and ultimately the adult organism. ESCs depend on various  
21  
22 signals for self-renewal: mouse-ESCs rely on the cytokine Leukemia Inhibitory Factor (LIF) and  
23  
24 signals from Bone Morphogenic Proteins (BMPs), which reinforce the pluripotency network and  
25  
26 block progression to lineage-commitment (Fig. 2A), whereas human-ESCs rely on Fibroblast  
27  
28 Growth Factor (FGF) signaling and a balance between Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) and  
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30 BMP-signaling (Fig. 2B) [3, 14, 51]. YAP and TAZ are important for regulating ESC self-renewal  
31  
32 and differentiation. The importance of YAP/TAZ during embryogenesis was uncovered when the  
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34 transcription factor TEAD4 was found to be critical for the induction of Cdx2, which is a  
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36 transcription factor required for the development of the trophectoderm-lineage, the outer cells of the  
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38 blastocyst stage embryo [52, 53]. However, TEAD4 expression is not restricted to the outer cells of  
39  
40 the blastocyst. YAP is found only in the nucleus in the outer cells; it is cytoplasmic in the ICM cells  
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42 and nuclear in the outer cells of the embryo, thus allowing TEAD4-mediated Cdx2 transcription in  
43  
44 these cells [54]. Although YAP is found in the cytoplasm in the ICM of mouse blastocyst, it is  
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46 found in the nucleus of ESCs, which are derived from the ICM. As ESCs differentiate, YAP nuclear  
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48 localization and protein levels diminish, and this is accompanied by increased Hippo pathway  
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50 activity [55]. The conditional knockout of TAZ in hESCs (but not YAP) and loss of YAP or TAZ in  
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52 mESCs result in loss of self-renewal and ESC pluripotency; overexpression of YAP prevents  
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54 differentiation in mESCs and results in increased reprogramming of fibroblasts to Induced  
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3 Pluripotent Stem Cells (IPSCs) [37, 55]. The latter are differentiated cells reprogrammed to an  
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5 ESC-like state by inducing the activity of four transcription factors (Oct-4, Sox-2, Klf-4 and cMyc)  
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7 [55, 56]. IPSCs can self-renew, they are indistinguishable from ESCs in several ways; therefore,  
8  
9 they are considered a valuable resource for cell or tissue replacement therapy. However, these data  
10  
11 suggest that YAP activity enhances the reprogramming process, in conjunction with Oct4, Sox2 and  
12  
13 Klf4. A possible mechanism may be via the Yes-Kinase-mediated phosphorylation of YAP, which  
14  
15 induces a YAP/TEAD2-gene expression program. YAP/TEAD2 directly bind to promoters and  
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17 activate the transcription of important stemness genes, such as Sox-2, Oct-4 and Nanog [55].  
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19 YAP/TAZ contribute to maintaining mESC pluripotency *in vitro* by mediating the BMP-induced  
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21 transcriptional program: the BMP signaling pathway leads to the activation of Smad1 transcription  
22  
23 factor and the recruitment of YAP to Smad1 on target gene promoters, enhancing Smad1 activity  
24  
25 [36]. In hESCs, TAZ binds Smad2/3-Smad4 heterodimers in response to TGF- $\beta$  stimulation; TAZ  
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27 depletion impairs Smad2/3-Smad4 accumulation in the nucleus and transactivation activity, and the  
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29 cells tend to differentiate [37, 50].  
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### 38 *SKIN STEM CELLS (SSCs)*

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40 To regenerate continuously and maintain its structural and functional integrity, the skin relies on the  
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42 self-renewing abilities of epidermal SCs residing in the basal layer of epidermis. Asymmetric  
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44 divisions in this SC compartment produce short-lived progenitor cells that stratify, leave the basal  
45  
46 layer, and move up through the suprabasal layers to the outer surface of the skin, as they terminally  
47  
48 differentiate. YAP plays an important role in epidermal development and SC homeostasis [3, 51].  
49  
50 Recent studies have highlighted that YAP overexpression causes a severe thickening of the  
51  
52 epidermal layer and that this hyperplasia is driven by the expansion of undifferentiated  
53  
54 interfollicular SCs and progenitor cells; the knockout of YAP leads to an epidermal hypoplasia, and  
55  
56 this phenotype has been attributed to the gradual loss of epidermal stem/progenitor cells and their  
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3 limited capacity to self-renew [3, 27]. In the skin, YAP is not regulated by the canonical Hippo  
4  
5 kinases, but rather by  $\alpha$ -Catenin, a component of Adherens Junctions (AJs), which is an upstream  
6  
7 negative regulator of YAP. These AJs could act as “molecular biosensors” of cell density and  
8  
9 positioning according to the “crowd control molecular model”: sensing increased cell density leads  
10  
11 to inhibition of SC expansion by inactivating YAP, whereas low basal cell density translates into  
12  
13 nuclear YAP localization and cell proliferation [48, 49].  
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### 20 *SMALL INTESTINE STEM CELLS (ISCs)*

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26 The intestinal epithelium is one of the most rapidly regenerating tissues in the body, turning over  
27  
28 completely every 4–5 days through the continual proliferation of intestinal stem cells located at the  
29  
30 base of the crypt (Crypt Base Columnar (CBC) cells, also known as Lgr5(+) cells) and at the “+4  
31  
32 position” relative to the crypt bottom [51]. The major factor promoting the self-renewing capacity  
33  
34 of Lgr5+ ISCs in mammals is the Wnt-pathway. The driving force behind Wnt-signaling is  $\beta$ -  
35  
36 Catenin. Generally, in a Wnt-unstimulated cell, Glycogen Synthase Kinase-3 (GSK-3)  
37  
38 phosphorylates the cytoplasmic pool of  $\beta$ -Catenin and promotes its degradation through an  
39  
40 ubiquitin-mediated proteasome pathway (Fig. 2C, left). In a Wnt-stimulated cell, the Wnt receptor  
41  
42 Frizzled activates Disheveled (Dsh), which in turn inhibits GSK-3 activity. On its inhibition, GSK-3  
43  
44 no longer phosphorylates  $\beta$ -Catenin, which accumulates in the cytosol. Stable  $\beta$ -Catenin  
45  
46 subsequently enters the nucleus, forms a transcriptional complex with members of the Lef/Tcf  
47  
48 family of DNA-binding proteins and regulates downstream target genes (Fig. 2C, right) [57].  
49  
50 Recently, the Hippo pathway together with its final effector YAP, have been found to be critical in  
51  
52 regulating ISC self-renewal and differentiation. YAP is found in the nucleus of ISCs and some other  
53  
54 crypt cells but is primarily cytoplasmic in the upper crypt and villi, where it is likely that Hippo  
55  
56 targets YAP for phosphorylation and consequent inhibition [3, 51]. The phosphorylation of  
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3 YAP/TAZ and their cytoplasmic localization counteracts Wnt-activity. Overexpression of active  
4  
5 YAP or conditional knockout of Mst/Sav-1 expands progenitor-like cells and blocks differentiation  
6  
7 [58, 59]. The aberrant proliferation induced by unphosphorylated YAP in ISCs is in part or wholly  
8  
9 due to the hyperactivation of Wnt-signaling because of enhanced  $\beta$ -Catenin transcriptional activity.  
10  
11 Specifically, when YAP and TAZ are phosphorylated by the Hippo pathway and sequestered in the  
12  
13 cytoplasm, phosphorylated YAP/TAZ interact with Dsh and  $\beta$ -catenin (Fig. 2C, right). Cytoplasmic  
14  
15 YAP inhibits the nuclear translocation of Dsh and  $\beta$ -catenin (through a mechanism which is distinct  
16  
17 from the degradation pathway), whereas cytoplasmic TAZ inhibits the activity of the degradation-  
18  
19 complex member Casein Kinase 1 (CK1), blocking Dsh phosphorylation. In the Wnt-ON state, if  
20  
21 phosphorylated YAP/TAZ is lost, or Hippo signaling is ablated, cells undergo hyperactivation of  
22  
23 Wnt-signaling owing to increased Dsh phosphorylation and/or nuclear accumulation as well as  
24  
25 additional nuclear  $\beta$ -catenin (Fig. 2D). Once in the nucleus, Dsh acts as a transcriptional co-factor  
26  
27 to induce  $\beta$ -catenin target genes in conjunction with another transcriptional co-factor cJUN [56]. In  
28  
29 addition, YAP interacts with  $\beta$ -catenin and TEAD in the nucleus to activate the expression of  
30  
31 proliferation-related genes. In conclusion, when the Wnt pathway is predominant, compared with  
32  
33 phosphorylated YAP, Wnt produced by Paneth cells (components of the intestinal stem cell niche)  
34  
35 and other sources is detected by ISCs in intestinal crypts: the ISCs divide and cells progress upward  
36  
37 out of the crypt and begin to differentiate. If YAP becomes overabundant in the cytoplasm of the  
38  
39 crypt cells, Wnt signaling is repressed, and the ISC niche is disrupted. This causes aberrant upward  
40  
41 migration of Paneth cells and loss of ISCs. Because of the ISC loss, the intestinal epithelium  
42  
43 degenerates.  
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### 53 *NEURAL, LIVER AND CARDIAC MUSCLE STEM CELLS*

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56 Neural progenitor cells reside along the sub-ventricular zone in the developing vertebrate neural  
57  
58 tube and are responsible for generating the myriad of cell types comprising the mature central  
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3 nervous system. The conditional knockout of Mst/Lats or YAP-activation expands neural progenitor  
4  
5 cells in the neural tube [3, 51, 60]. In the cerebellum, endogenous YAP is highly expressed in  
6  
7 Cerebellar Granule Neural Precursors (CGNPs). YAP overexpression expands CGNPs in the  
8  
9 cerebellum and leads to medulloblastoma. The CGNPs rely on Sonic-Hedgehog (Shh)-signaling to  
10  
11 expand and Shh-signaling induces the expression and nuclear localization of YAP, which then  
12  
13 drives the proliferation of these cells [61].  
14  
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16  
17 The adult liver has a distinctive ability to rapidly regenerate following acute injury. The  
18  
19 regeneration of the organ is dependent on the ability of hepatocytes and cholangiocytes (bile duct  
20  
21 cells) to proliferate and on heterogeneous populations of transit-amplifying bipotential progenitor  
22  
23 cells known as “oval cells” in rodents [62]. YAP overexpression leads to a dramatic but reversible  
24  
25 liver hyperplasia, caused by an exacerbated proliferation of mature hepatocytes [58]. Conversely,  
26  
27 conditional loss of YAP function leads to impaired liver function, primarily because of accelerated  
28  
29 hepatocyte turnover due to enhanced apoptosis. The conditional knockout of Mst1-2 leads to liver  
30  
31 overgrowth, with mixed hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC)  
32  
33 phenotypes, whereas conditional knockout of Sav-1 leads to a similar liver overgrowth and  
34  
35 development of HCC/CC mixed tumors, but shows increased number of oval cells without  
36  
37 concomitant hepatocyte-expansion [62-64].  
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43 In contrast to that in tissues such as the liver, the role of Hippo signaling in the heart is just  
44  
45 beginning to be delineated. Sav1/Lats2/Mst1-2 conditional knockout or YAP overexpression  
46  
47 promotes cardiomyocyte proliferation, resulting in embryos displaying a cardiomegaly phenotype,  
48  
49 whereas YAP conditional knockout leads to myocardial hypoplasia [51, 65]. YAP interacts with  $\beta$ -  
50  
51 Catenin in the nucleus to promote Wnt-signaling (a well-known promoter of cell-stemness and  
52  
53 proliferation in the heart), thus enhancing neonatal cardiomyocyte proliferation [65]. Moreover,  
54  
55 YAP indirectly promotes Wnt-signaling through the activation of the Insulin-like Growth Factor  
56  
57 (IGF) pathway (a potent signaling system that stimulates growth and blocks apoptosis in many  
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3 different cell types), resulting in the inactivation of GSK3 $\beta$  and consequently the Wnt degradation  
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5 complex [66].  
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### 10 11 **Structural and inhibition studies**

#### 12 13 *YAP/TAZ/Yki DOMAINS ORGANIZATION*

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17 The human YAP gene is located at 11q13; it can be transcribed into at least 4 isoforms (YAP1-4),  
18  
19 which are generated by differential splicing of short exons located within the transcriptional  
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21 activation domain of YAP: isoforms 1, 2, 3 and 4 have 504, 450, 488 and 326 residues in length,  
22  
23 respectively (Fig. 3, Table 3) [67-70]. YAP was originally identified in chicken as an interacting  
24  
25 protein of Yes protein tyrosine kinase. The interaction was shown to be mediated by the SH3-  
26  
27 domain of the Yes protein and the (Pro)-rich region (PVKQPPPLAP) of YAP (SH3 binding  
28  
29 domain, Fig. 3). Due to its size of 65 kDa, the chicken protein was referred to as YAP65 (Yes-  
30  
31 associated protein of 65 kDa). The human and mouse homologues were identified by using the  
32  
33 YAP65 cDNA to probe human and mouse cDNA libraries. During the course of sequence analysis  
34  
35 by comparing YAP with other proteins, a conserved module was observed in several proteins of  
36  
37 various species. This was named the WW-domain to reflect the sequence motif containing two  
38  
39 conserved and consistently positioned tryptophan (W) residues (Fig. 3). Two consecutive WW-  
40  
41 domains are present in all isoforms except isoform 2, which has only one WW domain. Isoform 3  
42  
43 containing 488 residues and two WW domains is the most thoroughly studied isoform. These WW-  
44  
45 domains are important for YAP to interact with transcription factors containing the PPXY-motif.  
46  
47 The N-terminal region harbors the TEAD-binding domain, containing HXRXXS-motifs (14-3-3  
48  
49 binding domain, Fig. 3). Phosphorylation of S127 within this motif creates a binding site for 14-3-3  
50  
51 proteins and results in YAP-cytoplasmic sequestration and inactivation. The C-terminus of Yap  
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53 proteins exhibits strong transactivation property (Transcriptional activation domain, Fig. 3), and it  
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3 contains a PDZ-binding motif (FLTWL, Fig. 3) critical for nuclear translocation and binding to the  
4  
5 PDZ-domain of other regulator-proteins such as ZO2. YAP proteins also contain a coiled-coil  
6  
7 domain within the transcriptional binding domain (Fig. 3) [69].  
8  
9

10 The TAZ gene can be transcribed into three variants, all of which have the same coding region for a  
11  
12 protein having 400 amino acids in length (Fig. 3, Table 3). TAZ, also referred to as WWTR1 (WW-  
13  
14 domain containing transcription regulator 1), is homologous to YAP3 with 42% amino acid  
15  
16 sequence identity and displaying similar domain organization but having only one WW-domain.  
17  
18 Biochemically, TAZ displays transcriptional co-activator function via interaction with PPXY-  
19  
20 containing transcriptional factors through its WW-domain. The C-terminal region is responsible for  
21  
22 the transcriptional co-activation property. Similar to YAP, TAZ has a C-terminus with a PDZ-  
23  
24 binding motif (FLTWL) and a N-terminal region, which harbors the TEAD-binding domain  
25  
26 containing the HXRXXS-motifs with the S89, whose phosphorylation creates a binding site for the  
27  
28 14-3-3 proteins, resulting in TAZ inactivation.  
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33 Both YAP and TAZ are homologous to fly Yki (Yorkie). Similar to YAP, Yki contains two WW-  
34  
35 domains. The N-terminal region of Yki shows the highest homology to YAP and TAZ, presenting  
36  
37 the Scalloped (Sd)-binding domain with S111 homologous to YAP-S127 and TAZ-S89.  
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#### 43 *YAP-TEAD COMPLEX: THE MAIN MEDIATOR OF YAP/TAZ/Yki TRANSCRIPTIONAL* 44 45 *FUNCTION* 46 47

48 TEADs in mammals and Sd in *Drosophila* are the major transcriptional factors mediating the  
49  
50 biological outcome of YAP/TAZ and Yki, respectively. YAP was identified as a tight binding and  
51  
52 major interacting protein for TEAD2 and was proposed to function as a general transcriptional co-  
53  
54 activator for the TEADs transcriptional factors. There are four related family members (TEAD1–4)  
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56 in mammals (Fig. 4, Table 4) [70]. The N-terminal regions of TEADs and Sd contain a conserved  
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3 TEA domain involved in recognizing DNA elements such as GGAATG in the promoter region of  
4 target genes. The NMR structure of the TEA domain (PDB ID: 2HZD) revealed a three-helix  
5 bundle fold with the helix 3 containing a bipartite Nuclear Localization Signal (NLS) [71]. The C-  
6 terminal regions of TEADs and Sd interact with YAP/TAZ and Yki, respectively. Mutation of  
7 specific YAP residues abolishes most, if not all, activity of YAP in promoting cellular  
8 transformation and mediating the transcriptional outcome, suggesting that interaction with TEADs  
9 is the major functional pathway of YAP. A similar functional relationship between TEAD and TAZ  
10 has been demonstrated: the TAZ residues essential for interacting with TEAD are also essential to  
11 induce transformation and are well conserved in YAP and Yki. The putative TEAD2-YAP Binding  
12 Domain (YBD) in a crystal structure of TEAD (PDB ID: 3L15) adopts an immunoglobulin IgG-like  
13 fold with two  $\beta$ -sheets packing against each other to form a  $\beta$ -sandwich [69]. One  $\beta$ -sheet contains  
14 five antiparallel strands, including  $\beta$ 1,  $\beta$ 2,  $\beta$ 5,  $\beta$ 8, and  $\beta$ 9, whereas the other contains seven parallel  
15 and antiparallel strands, including  $\beta$ 3,  $\beta$ 4,  $\beta$ 6,  $\beta$ 7, and  $\beta$ 10–12. In addition to the two major  $\beta$ -sheets,  
16 TEAD2-YBD contains two helix-turn-helix motifs that are absent in the IgG-fold. One helix-turn-  
17 helix motif consists of  $\alpha$ A and  $\alpha$ B and connects  $\beta$ 3 and  $\beta$ 4. This motif along with the  $\beta$ 2- $\beta$ 3 loop  
18 encircles the C-terminal  $\beta$ 12 strand, forming an unusual pseudoknot structure. The second helix-  
19 turn-helix motif consists of  $\alpha$ C and  $\alpha$ D and connects  $\beta$ 9 and  $\beta$ 10. This motif caps the opening at one  
20 end of the  $\beta$ -sandwich. There are several surface-exposed residues that are identical in TEAD-YBD  
21 from all species. All these conserved residues form a contiguous surface on one face of TEAD2-  
22 YBD that contains  $\beta$ 7,  $\alpha$ C,  $\alpha$ D, and on the back of the strands  $\beta$ 4,  $\beta$ 11, and  $\beta$ 12. The other face of  
23 TEAD2-YBD contains few conserved residues. Conserved residues are essential to shape the YAP  
24 binding pocket: the strands  $\beta$ 4,  $\beta$ 11, and  $\beta$ 12 form the base of this pocket, whereas  $\beta$ 3,  $\alpha$ A, and  $\alpha$ D  
25 form the walls. The residues in the center of the pocket are hydrophobic, whereas several residues  
26 lining the periphery of the pocket are polar or charged, including E267, K277, K301, E404, N405,  
27 and E429. Therefore, despite having an extensive YAP-binding surface, this pocket on TEAD2-  
28 YBD has both hydrophobic and hydrophilic characters, and it represents a major anchoring point  
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3 for YAP. A conserved tyrosine in human TEAD1-YDB (Y421, corresponding to Y442 in TEAD2)  
4  
5 is mutated to histidine in patients with a rare eye disorder called Sveinsson's chorioretinal atrophy  
6  
7 [30]. This mutation disrupts the YAP-TEAD interaction, thus hindering YAP-dependent induction  
8  
9 of proliferation. The crystal structure of the complex between human TEAD1 and YAP (PDB ID:  
10  
11 3KYS) confirmed the structure of the putative YBD and shed light on the structural features of the  
12  
13 YAP-TEAD interaction [13]. In this structure, YAP sequesters TEAD in a manner akin to a pair of  
14  
15 forceps, with three major interaction interfaces. A  $\beta$  strand in YAP (residues 52 to 57) represents  
16  
17 the first structural motif interacting with TEAD1  $\beta$ 7, through a series of H-bonds. The conserved  
18  
19 hydrophobic residues (61 to 73) of helix  $\alpha$ 1 in YAP represent the second contact area. An unusual  
20  
21 twisted-coil region in YAP (residues 86-100), also referred to as  $\Omega$ -loop motif, fits snugly within a  
22  
23 hydrophobic site on the TEAD1 surface [13]. The last two regions are connected, in YAP, through a  
24  
25 linker characterized by a PXX $\Phi$ P motif (residues 81-85). Similar to YBD in TEADs proteins, YAP,  
26  
27 TAZ and Yki conserve, in different species, the residues necessary for interaction with TEAD and  
28  
29 therefore for the growth promoting activity of the YAP oncogene. In the crystal structure of mouse  
30  
31 TEAD4-YAP (PDB ID: 3JUA), the major interactions of the last two interfaces are conserved,  
32  
33 whereas the  $\beta$  strand has not been resolved, and the  $\Omega$ -loop is referred to as a second alpha helix  
34  
35 ( $\alpha$ 2) [72]. It appears that all three sites of interaction act in concert to mediate the YAP-TEAD  
36  
37 complex;  $\beta$  strand, helices  $\alpha$ 1 and  $\Omega$ -loop/ $\alpha$ 2 in YAP appear to contribute most significantly to the  
38  
39 interaction with TEAD, whereas the PXX $\Phi$ P-containing loop appears to play a minor role. The  
40  
41 second interaction interface involves the helix  $\alpha$ 1 of YAP and the helices  $\alpha$ 3 and  $\alpha$ 4 of TEAD,  
42  
43 where the residues L65, L68 and F69 of YAP form the LXXLF motif interacting with the  
44  
45 hydrophobic groove formed by the residues F329, Y361, F365, K368, L369, L372, V381 and F385  
46  
47 of TEAD; all residues involved in this contact area are highly conserved in YAP and TEADs (Fig.  
48  
49 5, left panel). The third interaction site is primarily mediated by the  $\Omega$ -loop of YAP, which fits in  
50  
51 the pocket formed by  $\beta$ 4,  $\beta$ 11,  $\beta$ 12,  $\alpha$ 1 and  $\alpha$ 4 of TEAD1. The interaction of the YAP  $\Omega$ -loop with  
52  
53 TEAD1 is chiefly mediated by hydrophobic interactions: M86, L91, F95 of YAP establish Van der  
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3 Waals contacts with I262, V257, L297 and V406. This interaction is then strengthened by the polar  
4 contacts of K89 and S94 of YAP with D264 and E254 and Y421 of TEAD, respectively (Fig. 5,  
5 right panel). Although the sequence of TAZ lacks the PXXΦP motif, a computational model of its  
6 structure has suggested that its residues 24-56 can form both a  $\alpha$ -helix and a  $\Omega$ -loop that can  
7 adopt a conformation reproducing the spatial arrangement of the  $\alpha$ 1 and  $\Omega$ -loop of YAP,  
8 respectively [70, 72].  
9

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12 Recently, the crystal structures of murine Vgll1 and Vgll4 complexed with TEAD4 have been  
13 elucidated, uncovering the important role of Vestigial-like proteins 1-4 (Vg in *Drosophila*) as co-  
14 transcriptional factors. Similar to YAP and TAZ, Vgll proteins exert their function by binding the  
15 C-terminal region of TEAD proteins through the Tondu domain(s) (TDU). Mouse Vgll1-TEAD4  
16 crystal structure (PDB ID: 4EAZ) [73] revealed that Vgll1 interacts with TEAD through two  
17 structural elements: the first interaction interface is composed by hydrogen bonds formed by a  $\beta$   
18 strand of Vgll1 ( $\beta$ 2), interacting with  $\beta$ 7 of TEAD; the second interface is mediated by hydrophobic  
19 interactions between Vgll1 helix  $\alpha$ 1 (TDU domain) and TEAD helices  $\alpha$ 3 and  $\alpha$ 4. As revealed by  
20 the superposition of the respective crystallographic complexes, mVgll1 and YAP bind to  
21 overlapping regions of TEAD, highlighting similarities and differences between the binding modes  
22 of the proteins. The  $\beta$ 2 strand of mVgll1 and the  $\beta$ 1 of YAP occupy the same region, whereas  $\alpha$ 1 of  
23 mVgll1 is superposed to hYAP  $\alpha$ 1, sharing a short hydrophobic motif (V41, F45, A48 in mVgll1  
24 and L65, F69, V72 in hYAP) that interacts with the groove formed by TEAD  $\alpha$ 3 and  $\alpha$ 4 (Fig. 6). In  
25 contrast, mVgll1 lacks the  $\Omega$ -loop, which is fundamental for YAP/TAZ binding to TEAD-YBD  
26 surface [13]. Mutagenesis and TR-FRET experiments to describe the interaction modes of Vgll1  
27 fragments and hTEAD4 [74] have revealed that the peptide fragment formed by  $\beta$ 2 and  $\alpha$ 1 is  
28 fundamental for mVgll1 binding, showing nanomolar affinity for hTEAD4. This was unexpected,  
29 as YAP fragments missing the  $\Omega$ -loop lack most of their affinity for TEAD [13]. TR-FRET studies  
30 additionally showed that mVgll1-derived peptides can compete with hYAP, which supports the  
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3 hypothesis that YAP and Vgll have mutually exclusive effects on TEAD-dependent gene  
4 transcription. This hypothesis was further supported by a recent study, which clarified the function  
5 of Vgll4 and reported the crystallization of the mVgll4-TEAD4 complex (PDB ID: 4LN0) [75].  
6  
7 This crystal structure highlights striking differences between mVgll1 and mVgll4.  
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#### 10 11 12 13 14 15 16 *INHIBITION STRATEGIES OF YAP/TAZ/Yki*

17  
18 Considering the strict correlation between YAP hyperactivation and the outbreak of several  
19 malignancies, the modulation of its functions represents an innovative and promising approach to  
20 prevent and treat human cancers. Although the lack of information about the complete YAP  
21 structure prevents the application of a structure-based approach to discover novel compounds  
22 binding to YAP, the available structural data indicate that the YAP/TEAD complex is a suitable  
23 target for developing new cancer therapeutics. The TEAD surface should be druggable through the  
24 fragment-based strategy, and a battery of diverse compounds can be envisioned, which could dock  
25 into one or any combination of the three binding sites to disrupt or weaken the YAP-TEAD  
26 complex. Even if the design of small-molecule inhibitors of protein-protein interactions have  
27 traditionally represented a significant challenge, the availability of crystal coordinates for the YAP-  
28 TEAD complexes along with recent successes in structure-based design of protein-protein  
29 inhibitors allow to envision a more optimistic scenario for future accessibility to novel  
30 pharmacological tools interfering with YAP-mediated TEAD activation.  
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48 To identify small molecules that modulate YAP-dependent transcription by computer-aided drug  
49 design, Sudol et al. evaluated the possibility of targeting the WW domain, which recognizes proline  
50 rich motifs (PPXY), fundamental for YAP interaction with LATS and for its transcriptional activity  
51 [67]. Previously, the same group had predicted by virtual models that the cardiac glycoside  
52 digitoxin (Fig. 7D) could be considered as a putative ligand at the WW domain of dystrophin [76].  
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3 Digitoxin is used to treat cardiac arrhythmias, where its apparent mechanism of action involves  
4 modulating the activity of the sodium-potassium ATPase transporter pump. The potential affinity of  
5 digitoxin for the WW-domain has emerged by a docking strategy developed to dock 287 FDA-  
6  
7 approved small molecule drugs with 35 peptide-binding proteins, including 15 true positives. The  
8  
9 selection of peptide binding domains included twenty co-crystal structures selected from the Protein  
10  
11 Data Bank (PDB), representing a subset of eukaryotic linear motif (ELM) peptide binding domains  
12  
13 complexed with peptides. Regarding the true positives selection, fourteen co-crystal structures and  
14  
15 one NMR model were selected from the PDB, with the requirements that they have a protein and a  
16  
17 small molecule mimicking a natural peptide in the complex. A combined ligand and target  
18  
19 normalization procedure was performed to improve the ability to rank true positives. The docking  
20  
21 energy score was combined with a score based on the number of similar interactions formed  
22  
23 between the compound and the native ligand. The 20 top ranking hits included 6 true positives,  
24  
25 including digitoxin. Considering the similarities between YAP and dystrophin, Sudol et al. built a  
26  
27 homology model of YAP WW domain and hypothesized that digitoxin may bind the portion  
28  
29 recognizing the PPXY motif [67]. Thus, digitoxin was docked to the canonical hydrophobic groove  
30  
31 within the WW-domain of YAP. Digitoxin could engage in an extensive network of intermolecular  
32  
33 Van der Waals and hydrogen bonding contacts with an array of residues, such as Y188, L190, T197  
34  
35 and W199, lining the hydrophobic groove within the WW domain. Moreover, these residues are  
36  
37 critical for the binding of PPXY ligands. However, other residues such as H192 and Q195 within  
38  
39 the WW-domain, which also play a key role in the binding of PPXY ligands, do not appear to be  
40  
41 important for binding digitoxin. This suggests that digitoxin is unlikely to target all WW-domains  
42  
43 indiscriminately and that potential opportunities exist for the chemical modification of digitoxin to  
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45 enhance its specificity toward a small group of WW-domains involved in regulating a specific  
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47 signaling cascade such as the Hippo pathway. Currently, this is only a speculative hypothesis and  
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49 no experimental data regarding the affinity of digitoxin for YAP are available.  
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3 Recent studies have highlighted the capability of G-protein coupled receptor (GPCR) signaling to  
4 regulate the Hippo pathway in a manner that is dependent on the specific G-protein coupled to the  
5 receptor [11, 77]. This discovery arose from the observation that in multiple cell lines, YAP is  
6 highly phosphorylated under serum starvation, whereas the addition of serum results in a rapid  
7 decrease in YAP phosphorylation. This suggested the presence of a serum component that activates  
8 YAP by inducing its dephosphorylation and nuclear localization. Further studies demonstrated that  
9 the active ingredient in serum was an amphiphilic molecule with an acidic group, such as  
10 lysophosphatidic acid (LPA) or sphingosine-1-phosphate (S1P). LPA and S1P act via activation of  
11 G12/13- or Gq/11-coupled receptor signaling, which leads to inhibition of Lats1/2 kinases and  
12 subsequent activation of YAP [77, 78]. In contrast, treatment of the same cell lines with epinephrine  
13 and glucagon, which are known Protein-Kinase A (PKA) activators, increases YAP phosphorylation,  
14 via activation of the Gs-coupled receptors, which stimulate adenylyl cyclase (AC). This results in  
15 the accumulation of cAMP, an important second messenger with diverse physiological functions,  
16 including cell proliferation and differentiation. Thus, cAMP acts through protein kinase A (PKA) to  
17 stimulate Lats kinase activity and inhibit YAP/TAZ. Altogether these data underline the possibility  
18 to modulate YAP/TAZ by a wide range of extracellular signals via GPCRs.  
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39 In a recently registered patent, Kung-Lian Guan et al. report the results of a HTS strategy based on a  
40 reporter assay in a mammalian cell culture system, consisting of a luciferase reporter and a Gal4-  
41 fused TEAD transcription factor [79]. A collection of small molecules was screened to search for  
42 potential compounds that regulate YAP, thus leading to changes in the TEAD-dependent expression  
43 of the luciferase-reporter. A particular chemical compound (CI08) was identified, which is an oxime  
44 derivative of 9H-Fluoren-9-one bearing two piperidinyl-sulfonyl groups (Fig. 7C). This compound  
45 potently inhibits YAP by promoting its ubiquitination and subsequent proteasome-mediated  
46 degradation. It also inhibits cell proliferation and retards the migration of multiple cancer cell lines  
47 *in vitro*, demonstrating its capability to inhibit YAP activity by decreasing the YAP protein levels in  
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3 a cell-type-independent manner. Anti-tumor potential of CI08 has also been evaluated in a xenograft  
4  
5 mouse model, showing that CI08 blocks melanoma and lung adenocarcinoma tumor growth and  
6  
7 induces apoptosis in cancer cells.  
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10 Liu-Chittenden et al. set up a luciferase reporter assay to test the YAP-dependent transcriptional  
11  
12 activity of a Gal4-TEAD4 assembly and screened the Johns Hopkins Drug Library to identify  
13  
14 molecules that inhibit the YAP-TEAD interaction. Verteporfin (VP, trade name: Visudyne by  
15  
16 Novartis) and protoporphyrin IX (PPIX) have been identified as top hits of the screening (Fig. 7A).  
17  
18 VP and PPIX are compounds belonging to the porphyrin family, which are aromatic heterocyclic  
19  
20 molecules composed of four modified pyrrole units, interconnected at their  $\alpha$ -carbon atoms via  
21  
22 methine bridges [29]. Co-immunoprecipitation assays revealed that both PPIX and VP inhibit the  
23  
24 YAP-TEAD complex formation at 10  $\mu$ M, whereas VP showed >50% inhibition at 2.5  $\mu$ M,  
25  
26 showing a higher potency than PPIX. VP is used clinically as a photosensitizer in the photodynamic  
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28 therapy of neovascular macular degeneration, where it is activated by laser light to generate reactive  
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30 oxygen radicals that eliminate the abnormal blood vessels. As an inhibitor of the YAP-TEAD  
31  
32 interactions, however, it does not require light activation. It was determined that VP selectively  
33  
34 binds YAP, thus altering YAP conformation and abrogating its interaction with TEAD *in vitro*.  
35  
36 Moreover, it was also demonstrated that VP inhibits the oncogenic activity of YAP *in vivo*: VP  
37  
38 suppresses the liver overgrowth resulting from either YAP overexpression or activation of  
39  
40 endogenous YAP [29]. Recently, the effects of VP without light activation have been evaluated on  
41  
42 human retinoblastoma cell lines [80]. This study shows that VP determines inhibition of cell growth  
43  
44 and viability and that it interferes with the YAP-TEAD proto-oncogene pathway in human  
45  
46 retinoblastoma cells. These results are encouraging because VP is a clinically applied drug with few  
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48 side effects. Moreover, considering that *in vivo* results were obtained using an aqueous preparation  
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50 in which VP bioavailability is sub-optimal, compared with the lipid-based formulation used in  
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3 Visudyne, this strategy may represent an excellent therapeutic approach with minimal adverse  
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5 effects.  
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7  
8 Very recently, Zhang et al. made an important headway in the study of the YAP–TEAD complex,  
9  
10 discovering a potent cyclic peptide, which inhibits the protein–protein complex by mimicking the  $\Omega$   
11  
12 loop of YAP [81]. Truncation studies and an alanine scan performed on the TEAD-binding domain  
13  
14 of YAP provided information about the optimal length of the synthetic peptide and advantageous  
15  
16 mutations to obtain active parent peptides of YAP. Moreover, the authors optimized the peptide by  
17  
18 applying conformational constraints to the structure. As observed in the YAP–TEAD co-crystal  
19  
20 structure, 3KYS, R87 and F96 of YAP are kept in close contact, within the  $\Omega$  loop, by a cation- $\pi$   
21  
22 interaction. The alanine scan study indicated that they are important for forming the complex, even  
23  
24 if these residues are not involved in a direct interaction with TEAD surface. This suggested that  
25  
26 R87 and F96 have a critical role in maintaining YAP in its active conformation. The authors thus  
27  
28 applied a macrocyclization strategy, replacing R87 with homocysteine and F96 with cysteine and  
29  
30 inducing the formation of a disulfide bond between the two new residues. The inhibitory activity of  
31  
32 the peptide was improved through several beneficial mutations: replacement of M86 with 3-Cl-  
33  
34 phenylalanine, mutation of L91 to norleucine and of D93 to alanine resulted in a cyclic peptide with  
35  
36  $IC_{50}$  equal to 0.025  $\mu$ M, nearly 1500-fold more potent than the parent YAP<sup>84–100</sup> peptide fragment,  
37  
38 which corresponds to the  $\Omega$  loop (Fig. 8). A computational model provided a rational explanation of  
39  
40 the high potency of the synthetic peptide, showing favorable Van der Waals interactions between  
41  
42 the 3-Cl-phenylalanine substituent and hydrophobic residues on TEAD surface. GST pull-down and  
43  
44 functional assays revealed that the synthetic cyclic peptide competes with the endogenous YAP  
45  
46 protein, showing a high affinity for TEAD. Altogether, this study reveals not only a potent inhibitor  
47  
48 of the YAP–TEAD interaction but also an exhaustive structure-activity relationship landscape for  
49  
50 the YAP  $\Omega$  loop. Additionally, an enhancement of the hydrophobic properties at M86, L91 and F95  
51  
52 of YAP represents a suitable strategy to improve the affinity of YAP-derived peptides for TEAD,  
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3 and polar interactions between S94 of YAP and Y421 and E255 of TEAD are fundamental. This  
4  
5 information can be advantageous for the design of small molecules inhibiting the YAP–TEAD  
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7 complex.  
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## 10 11 12 13 **CONCLUSIONS**

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16 Over the last few years, the Hippo pathway has emerged as a promising anticancer target, as  
17  
18 revealed by several experimental lines of evidence, which indicate that targeting the Hippo pathway  
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20 represents an effective strategy against oncogenic progression. It is becoming increasingly clear that  
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22 the Hippo pathway can regulate SC proliferation and maintenance; therefore, its modulation may be  
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24 therapeutically useful for tissue repair and regeneration following injury. Moreover, the complex  
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26 network of regulatory components within the Hippo pathway is being elucidated, and robust assays  
27  
28 that can measure the activity of this pathway have been established. Altogether, these findings offer  
29  
30 new possibilities for discovering useful pharmacological tools to further understand the precise role  
31  
32 of the Hippo pathway components and simultaneously design novel small molecules that modulate  
33  
34 the Hippo pathway. Although small molecules interfering with the Hippo pathway have been  
35  
36 reported, the molecular information is scarce and incomplete. For example, sphingosine-1-  
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38 phosphate (S1P) has been reported as a potent activator of YAP, promoting YAP nuclear  
39  
40 localization [78]. As revealed by siRNA transfection experiments, S1P determines YAP activation  
41  
42 through the Rho GTPase-mediated pathway, which is a well-known positive modulator of  
43  
44 YAP/TAZ. A recently published patent reports the results of a HTS campaign that led to the  
45  
46 discovery of compounds, including a compound in Fig. 7C, which promotes a GPCR-mediated  
47  
48 activation of the Hippo pathway and the consequent YAP phosphorylation and ubiquitination [79].  
49  
50 Recently, a small molecule (C19, 4-(4(3,4-dichlorophenyl)-1,2,5-thiadiazol-3-yloxy)butanol, Fig.  
51  
52 7B) has been observed to inhibit the Hippo pathway by activating Mst/Lats kinase and promoting  
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54 TAZ (but not YAP) phosphorylation and inactivation [82]. Furthermore, C19 markedly inhibited  
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3 tumor growth *in vivo*. However, the molecular site of action for these compounds is not precisely  
4  
5 characterized, which hampers structure-based discovery of new small molecules and their chemical  
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7 optimization. Currently, YAP/TAZ could be considered a promising target for this aim, considering  
8  
9 the availability of some structural information about their complex with TEAD. Although a potent  
10  
11 cyclopeptide that disrupts the YAP–TEAD complex formation has been recently discovered, the  
12  
13 only small molecule reported so far to directly inhibit the protein–protein interaction is verteporfin.  
14  
15 Despite the interest regarding this compound, which is already being used as a drug, detailed  
16  
17 structural information about its interaction with YAP or TEAD is still lacking, which signifies a  
18  
19 pivotal role to (virtual) screening campaigns and/or fragment-based approaches for the discovery of  
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21 new small-molecule inhibitors of YAP activation.  
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### 27 **Acknowledgments**

28  
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32  
33 (New molecules for the control and differentiation of stem cells).  
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Table 1: Hippo signaling pathway core components and modulators in *D. melanogaster* and mammals

<i>D. melanogaster</i> Modulators	Mammals	Role in the pathway	Effect on Yki/YAP
Ft (Fat)	Ft1-4	In <i>Drosophila</i> , Ft initiates signaling upon binding to Ds	-
Ds (Dachsous)	Dchs1-2	In <i>Drosophila</i> , Ds binds to and activates Ft	-
Ex (Expanded)	FRMD6 (Ex1)	In <i>Drosophila</i> , the Ex-Mer-Kibra complex activates Hpo	-
Mer (Merlin)	NF2	In <i>Drosophila</i> , the Ex-Mer-Kibra complex activates Hpo	-
Kibra	WWC1-2	In <i>Drosophila</i> , the Ex-Mer-Kibra complex activates Hpo	-
Crb (Crumbs)	Crb1-3	Apical polarity complex with PatJ and Std	-
PatJ	PatJ, MUPP1	Apical polarity complex with Crb and Std	
Std (Stardust)	Pals1	Apical polarity complex with Crb and PatJ	
aPKC	aPKC	Apical polarity complex with Par3 and Par6 (in <i>Drosophila</i> )	+
Par3	Par3	Apical polarity complex with Par6 and aPKC (in <i>Drosophila</i> )	
Par6	Par6	Apical polarity complex with Par3 and aPKC (in <i>Drosophila</i> )	
Lgl	Lgl1-2	Baso-lateral polarity complex	-
Scrib (Scribble)	Scrib	Baso-lateral polarity complex	
Dlg (Disc Large)	Dlg1-4	Baso-lateral polarity complex	
dRASSF		In <i>Drosophila</i> , dRASSF competes with Sav in binding to Hpo	+
	RASSF1-6	In mammals, RASSF activates Mst1-2 (except RASSF6)	-
dSTRIPAK	PP2A	Dephosphorylates Hpo/Mst1-2	+
Jub	Ajuba	Interacts with Sav/Sav1 and Wts/Lats1-2	+
Dco		In <i>Drosophila</i> , Dco enhances Ft activation	
	CK1 $\delta/\epsilon$	In mammals, CK1 $\delta/\epsilon$ primes YAP for degradation	-
	AMOT, AMOTL1, AMOTL2	Modulate YAP subcellular localization and/or phosphorylation	-
ZO-2	ZO1-2	Sequesters YAP/TAZ in the cytoplasm	-
	PTPN14	Sequesters YAP/TAZ in the cytoplasm	-
$\alpha$ -Catenin	$\alpha$ -Catenin	Stabilizes YAP-14.3.3 complex	-

Core components			
Hpo (Hippo)	Mst1-2	Phosphorylates and binds to Sav/Sav1; phosphorylates Mats/Mob1	-
Sav (Salvador)	Sav1	Forms a kinase complex with Hpo/Mst1-2; the complex phosphorylates Wts/Lats1-2	-
Wts (Warts)	Lats1-2	Forms a kinase complex with Mats/Mob1	-
Mats	MOBKL1A-B (Mob1)	Forms a kinase complex with Wts/Lats1-2; the complex phosphorylates Yki/YAP/TAZ	-
Yki (Yorkie)	YAP, TAZ	Transcriptional co-activator	
Sd (Scalloped)	TEAD1-4	Transcription factor	

Table 2: Known effects and mechanisms of action of YAP in different stem cell types

Stem Cell Type	YAP Activation	YAP Inactivation	Mechanism of Action
<b><u>Embryonic SCs</u></b>	Pluripotency support; Hindering of differentiation; Reprogramming process enhancement	Loss of self-renewal and pluripotency	Direct induction of important stemness-genes (i.e., Oct4, Sox2, Nanog)
<b><u>Skin SCs</u></b>	Thickening of epidermal layer; Hyper-keratinization; Tumor formation	Epidermal hypoplasia;	YAP overexpression drives the expansion of undifferentiated interfollicular SCs and progenitor cells;  YAP inactivation drives the gradual loss of epidermal stem/progenitor cells and their limited capacity to self- renewal
<b><u>Intestinal SCs</u></b>	ISCs proliferation and expansion of progenitor-like cells	Loss of ISCs and degeneration of the intestinal epithelium	Nuclear YAP enhances Wnt-signaling Cytoplasmic YAP represses Wnt- signaling
<b><u>Neural SCs</u></b>	Neural progenitor cells expansion; Expansion of cerebellar granule neural precursors; Medulloblastoma		Shh-signaling induces the expression and nuclear localization of YAP in cerebellar granule neural precursors
<b><u>Liver SCs</u></b>	Liver hyperplasia; hepatocyte hyperproliferation	Impaired liver function; hepatocyte apoptosis	YAP overexpression enhances proliferation of mature hepatocytes; YAP inactivation induces enhanced hepatocyte apoptosis
<b><u>Cardiac Muscle SCs</u></b>	Cardiomyocyte proliferation	Myocardial hypoplasia	Nuclear YAP enhances Wnt-signaling (both directly and via IGF-pathway);

Table 3: Percentage of identity among the four isoforms of hYAP and hTAZ

% identity	hYAP2	hYAP3	hYAP4	hTAZ
hYAP1	89	96	64	38
hYAP2		92	53	42
hYAP3			61	39
hYAP4				28

Table 4: Percentage of identity among the four isoforms of hTEAD

% identity	hTEAD2	hTEAD3	hTEAD4
hTEAD1	63	69	73
hTEAD2		61	61
hTEAD3			68



Figure legends:

**Figure 1.** The Hippo signaling pathway in mammals: the Hippo pathway is a kinase cascade consisting of serine/threonine phosphorylation events leading to cell proliferation inhibition and the promotion of apoptosis via inhibition of the transcriptional co-activators YAP/TAZ. The picture is a schematic representation of several interactors and regulators that play an important role in the mammalian Hippo pathway; pointed arrowheads represent activation, blunted arrowheads represent inhibition; dashed lines represent regulation mechanisms that are not fully elucidated (see text for detailed description).

**Figure 2:** (A) Self-renewal and differentiation regulation in mouse-ESCs: mESCs depend on the cytokine Leukemia Inhibitory Factor (LIF), signals from Bone Morphogenic Proteins (BMPs) and Wnt-signaling to reinforce the pluripotency network and block differentiation and progression to lineage-commitment. The LIF-pathway leads to the activation of JAK-STAT signaling, with STAT3 homodimers acting as transcription factors for major pluripotency and self-renewal genes; BMP-signaling determines the activation of Smad1/5-Smad4 transcription factors, targeting promoters for major stemness genes. (B) Self-renewal and differentiation regulation in human-ESCs: the self-renewal and differentiation of hESCs depend on Fibroblast Growth Factor (FGF) signaling and a balance between Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) and BMP-signaling. FGF-signaling supports the self-renewal via Protein Kinase C (PKC) and Phosphatidylinositol-3-Kinase (PI3K). TGF- $\beta$  signaling leads to Smad2/3 activation and the formation of Smad2/3-Smad4 heterodimer, which act as transcription factors promoting self-renewal and blocking differentiation. BMP-signaling leads to Smad1/5 activation and subsequent Smad1/5-Smad4 heterodimer formation, promoting differentiation and inhibiting self-renewal: hESCs fate depends on the balance between the two pathways. (C) Wnt-signaling in intestinal-SCs: Wnt-OFF state (left), Wnt-ON state (right); (D) Hippo/Wnt-signaling effects on intestinal crypt destiny: active YAP or Hippo ablation enhances Wnt-signaling, increasing Dsh phosphorylation and consequent  $\beta$ -catenin nuclear accumulation. Once in the nucleus, YAP interacts with  $\beta$ -catenin and TEAD to activate the expression of proliferation-related genes, and Dsh acts as a transcriptional co-factor to induce  $\beta$ -catenin target genes.

**Figure 3:** Alignment of the four isoforms of human YAP and human TAZ. Identical residues are highlighted as follows: red if identical among 3 out of 5 sequences, yellow if identical among 4 out of 5 sequences, and green if identical among all the 5 sequences. Protein domains are indicated as follows: TEAD-binding domain in magenta, 14-3-3-binding domain in red, WW domains in

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3 blue, SH3-binding domain in green, Transcriptional activation domain in orange, Coiled-coil  
4 domain in brown, PDZ-binding domain in purple.

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7 **Figure 4:** Alignment of the four isoforms of human TEAD. Identical residues are highlighted as  
8 follows: red if identical among 3 out of 5 sequences, yellow if identical among 4 out of 5  
9 sequences, and green if identical among all the 5 sequences. Protein domains are indicated as  
10 follows: DNA-binding TEA domain in orange, YAP-binding domain in magenta.

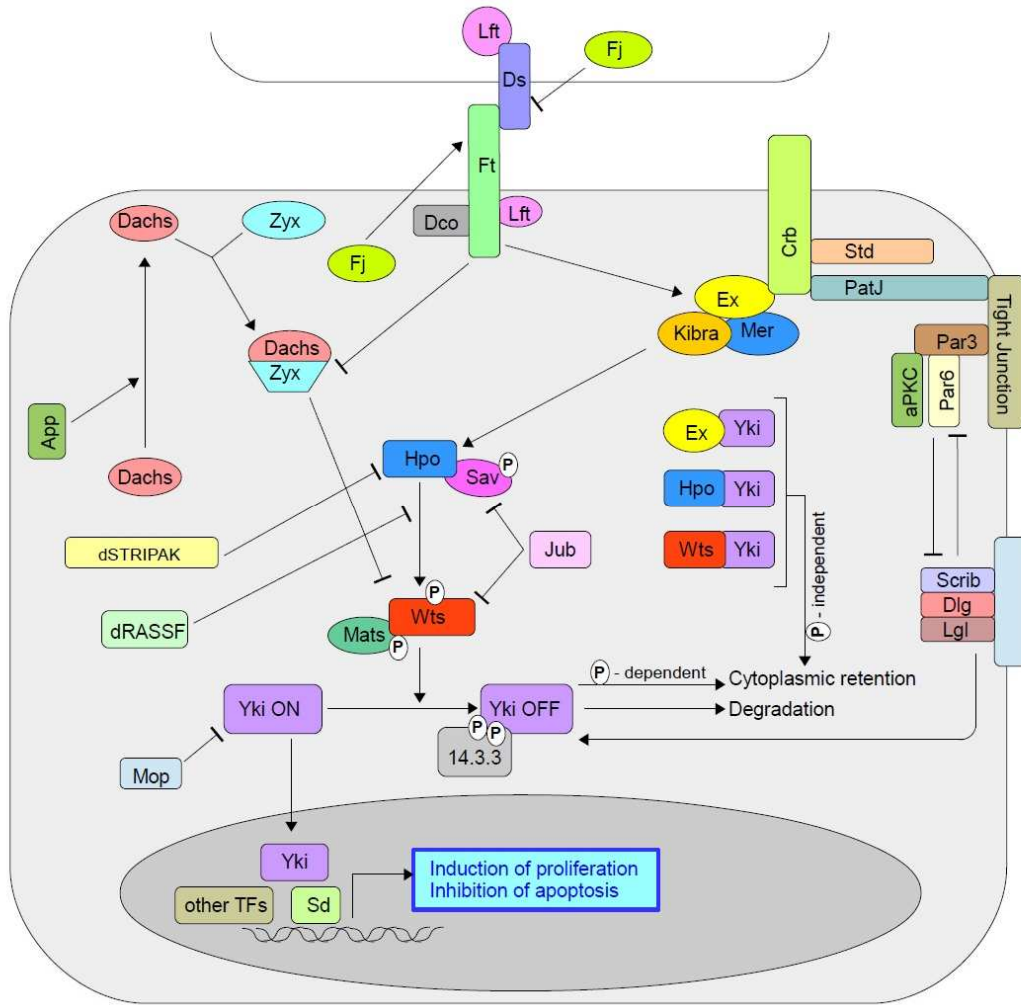
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14 **Figure 5:** Protein–protein interactions between human YAP (orange ribbons; residues 50–100  
15 from crystal structure 3KYS) and human TEAD (white ribbons; residues 200–426 from crystal  
16 structure 3KYS). Left panel: lipophilic residues on  $\alpha$ -helix 1 (orange carbons) of YAP interact  
17 with the hydrophobic groove formed by the  $\alpha$ -helix 3 and 4 of TEAD (blue ribbons). Right panel  
18 (resulting from 90° rotation around y axis): the interaction between the YAP  $\Omega$ -loop and TEAD is  
19 mediated through Van der Waals interactions between hydrophobic residues and through  
20 hydrogen bonds highlighted by yellow dots (YAP residues: orange carbons; TEAD residues: white  
21 carbons).

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28 **Figure 6:** Comparison of the interacting conformations of human YAP (orange ribbon; residues  
29 50–100 from crystal structure 3KYS) and mouse Vgll1 (cyan ribbons; residues 19–50 from crystal  
30 structure 4EAZ) with human TEAD (white surface; residues 200–426 from crystal structure  
31 3KYS).

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35 **Figure 7:** Chemical structures of small molecules interacting with Hippo pathway components are  
36 shown; (a) verteporfin (trade name Visudyne by Novartis) consists of a mixture of equally active  
37 regioisomers; verteporfin is a light-activated drug, used in photodynamic therapy for the treatment  
38 of macular degeneration. (b) C19 (4-(4(3,4-dichlorophenyl)-1,2,5-thiadiazol-3-yloxy)butanol). (c)  
39 2,7-bis(piperidin-1-yl-sulfonyl)-9H-fluoren-9-one oxime. (d) Cardio-active glycoside digitoxin.

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44 **Figure 8:** Chemical structure of a potent cyclopeptide inhibiting the YAP–TEAD interaction. The  
45 peptide mimics the YAP  $\Omega$  loop (residues 84–100); R87 and F96 were mutated to cysteine and  
46 homocysteine, respectively, to allow the formation of an internal disulfide bond; further  
47 modifications were performed to improve binding affinity to TEAD.  $\alpha$ -carbons of mutated  
48 residues are marked by an asterisk.

Figure 1:



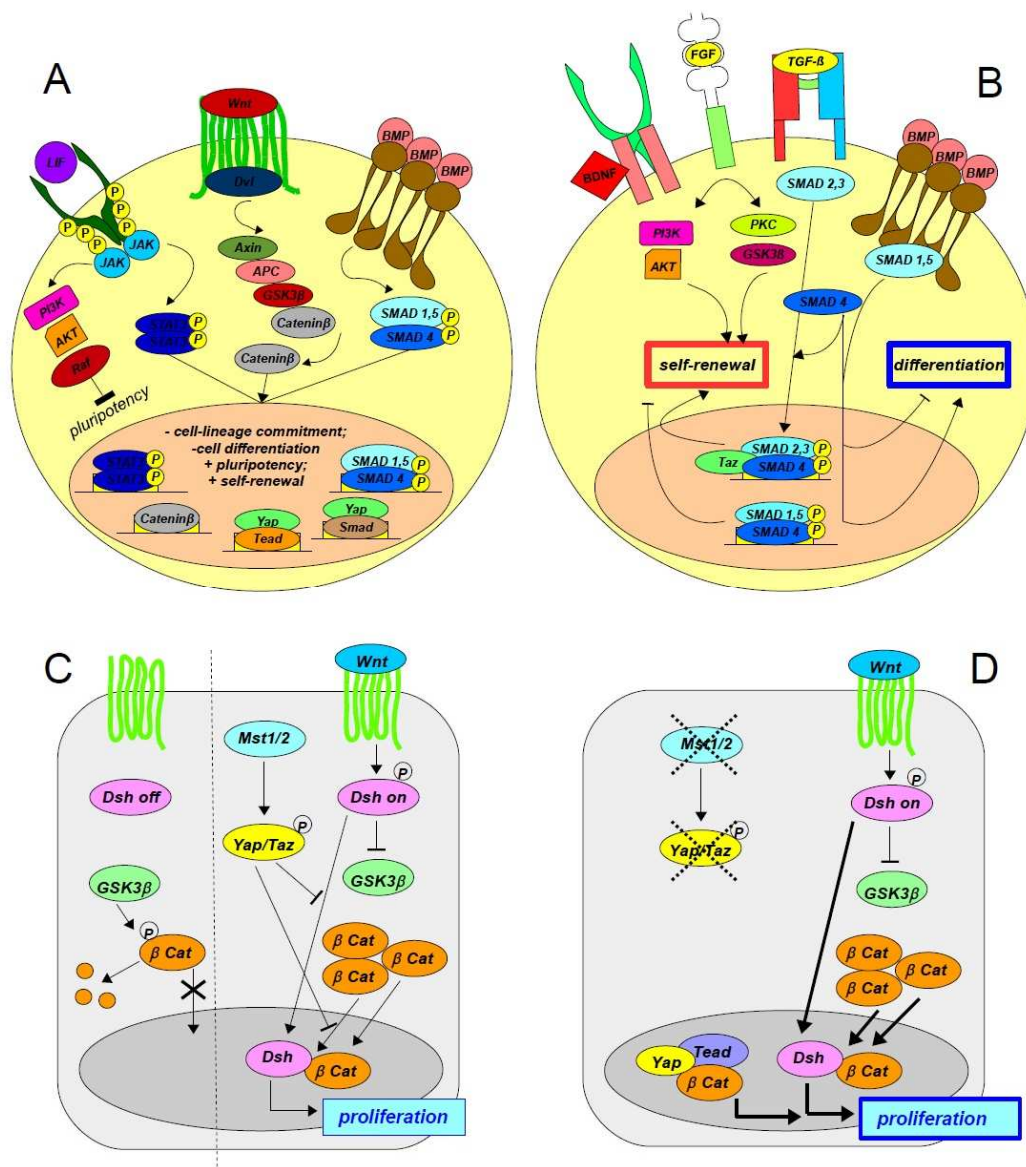


Figure 2

Figure 3:

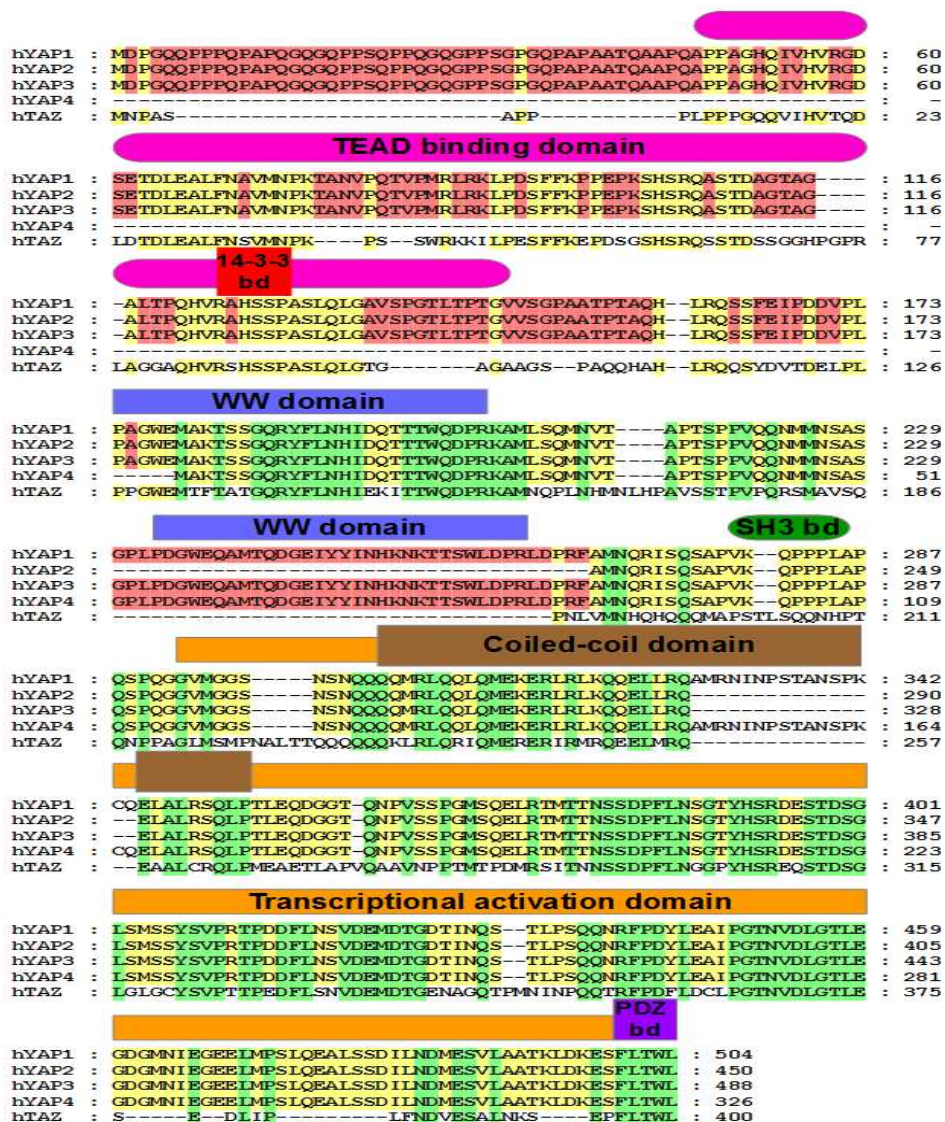




Figure 4:

TEAD1 : -----MEP--S~~W~~SGSESP-AENMERMSDSADK~~E~~IDNDAEGVWSPDIEQSFQEALAI : 49  
 TEAD2 : MGEPRAGAALDDGSGWTG~~S~~EEG-SEEGT~~G~~SEGAAGDGGPDAEGVWSPDIEQSFQEALAI : 59  
 TEAD3 : -----MAS--NSWNASSP-GEARE~~D~~GPEGLDKGLDNDAGVWSPDIEQSFQEALAI : 49  
 TEAD4 : M-EGTAGTITS--NEWSSPTSPEGSTAS~~G~~SQALDK~~F~~IDNDAEGVWSPDIEQSFQEALAI : 57

**DNA-binding TEA domain**

TEAD1 : YPPCGRRKI ILSDEGKMYGRNELIARY IKLRTGKTRTRKQVSSH IQVLARRKSRDFH~~S~~KL : 109  
 TEAD2 : YPPCGRRKI ILSDEGKMYGRNELIARY IKLRTGKTRTRKQVSSH IQVLARRKSR~~E~~IQSKL : 119  
 TEAD3 : YPPCGRRKI ILSDEGKMYGRNELIARY IKLRTGKTRTRKQVSSH IQVLARKK~~V~~REYQVGI : 109  
 TEAD4 : YPPCGRRKI ILSDEGKMYGRNELIARY IKLRTGKTRTRKQVSSH IQVLARRKAREIQAKL : 117

TEAD1 : K----DQ~~T~~AKDKALQHMAAMSSAQIVSATAIHNKLG~~L~~PG-I~~P~~RPTFPGAPGF~~W~~PMIQTG : 164  
 TEAD2 : K----DQ~~V~~SKDKAFQTMATMSSAQLISAPSLQAKLGPTG----PQASE~~L~~FQFW~~S~~GG--SG : 169  
 TEAD3 : KAMNLDQVSKDKALQSMASMSAQIVSASVLQNK~~F~~SP~~F~~SP~~L~~EQAVF~~T~~SSRFW~~S~~SP~~L~~LG : 169  
 TEAD4 : K----DQ~~A~~AKDKALQSMAMSSAQIIISATAFHSSMALAR---GPGRPAVSGFWQ~~G~~ALP-G : 169

TEAD1 : Q-PGSSQDVKPFVQQA~~Y~~PIQ--PAVTAP~~I~~PGFE-PAS-AP-APSVPA---WQGRSIGTTK : 215  
 TEAD2 : P-PWNVPDVKPF~~S~~QTPFTLSLTPPST-DLPGYEPQALSPLPPPT~~S~~PPAWQARGLGTAR : 227  
 TEAD3 : QQPGPSQ~~I~~KPPFAQ~~P~~AYPIQ--PPLPPTLSSYE-PLAPLP-SAAA-SVPVWQDR~~T~~IASSR : 224  
 TEAD4 : Q-AGTSHDVKPF~~S~~QTYAVQ--PPL--PLPGFES~~P~~AGPAP-SPSAPPAPFWQGRSVASSK : 223

**YAP binding domain**

TEAD1 : LRLVEFSAFLEQQ~~R~~DPDSYNKHLFVHIGHANHSYSDPLESVDIRQIYDKFPEKKGGLKE : 275  
 TEAD2 : LQLVEFSAFVEPPDAVDSYQRHLFVHISQHCPSGAPPLESVDV~~R~~QIYDKFPEKKGGLRE : 287  
 TEAD3 : LRLLEYSAFMEVQ~~R~~DPDTYSKHLFVHIGQTNPAFSDP~~P~~LEAVD~~V~~RQIYDKFPEKKGGLKE : 284  
 TEAD4 : LWMLEFSAFLEQQ~~D~~PD~~T~~YNKHLFVHIGQSSPSYSDPYLEAVDIRQIYDKFPEKKGGLKD : 283

TEAD1 : LFGKGPQNAFFLVKFWADLNCNIQ-DDAGA-----FYGVTSQYESS~~E~~NMTVTCSTKV : 326  
 TEAD2 : LYDRGPF~~H~~AFFLVKFWADLNWGPSGEEAGAGGSISSGGFYGVSSQY~~E~~LEHMTLTCSSKV : 347  
 TEAD3 : LYEKGP~~P~~NAFFLVKFWADLNSTIQ-EGPGA-----FYGVSSQYSSADSMTISVSTKV : 335  
 TEAD4 : LFERGPN~~A~~FFLVKFWADLNTNIE-DEGSS-----FYGVSSQYESS~~P~~ENMIITCSTKV : 334

TEAD1 : CSFGKQVVEKVETEYARFENGRFVYRINRSPMCEYMINFIHKLKHLPEKYM~~M~~NSVLENFT : 386  
 TEAD2 : CSFGKQVVEKVETERA~~Q~~LEDGRFVYRLLRSPMCEYLVN~~F~~LHKLRLQLPERYM~~M~~NSVLENFT : 407  
 TEAD3 : CSFGKQVVEKVETEYARLENGRFVYRIHRSPMCEYMINFIHKLKHLPEKYM~~M~~NSVLENFT : 395  
 TEAD4 : CSFGKQVVEKVETEYARYENGHYSYRIHRSPMCEYMINFIHKLKHLPEKYM~~M~~NSVLENFT : 394

TEAD1 : ILLVVTNRDTQETLLCMACVFEVSNSEHGAQH~~H~~IYRLVKD : 426  
 TEAD2 : ILQVVTNRDTQELLLCTAYVFEVSTSERGAQH~~H~~IYRLV~~R~~D : 447  
 TEAD3 : ILLQVVTNRDSQETLLVIAFVFEVSTSEHGAQH~~H~~VYKLVKD : 435  
 TEAD4 : ILLQVVTNRDTQETLLCIAVFEVSSASEHGAQH~~H~~IYRLVKE : 434

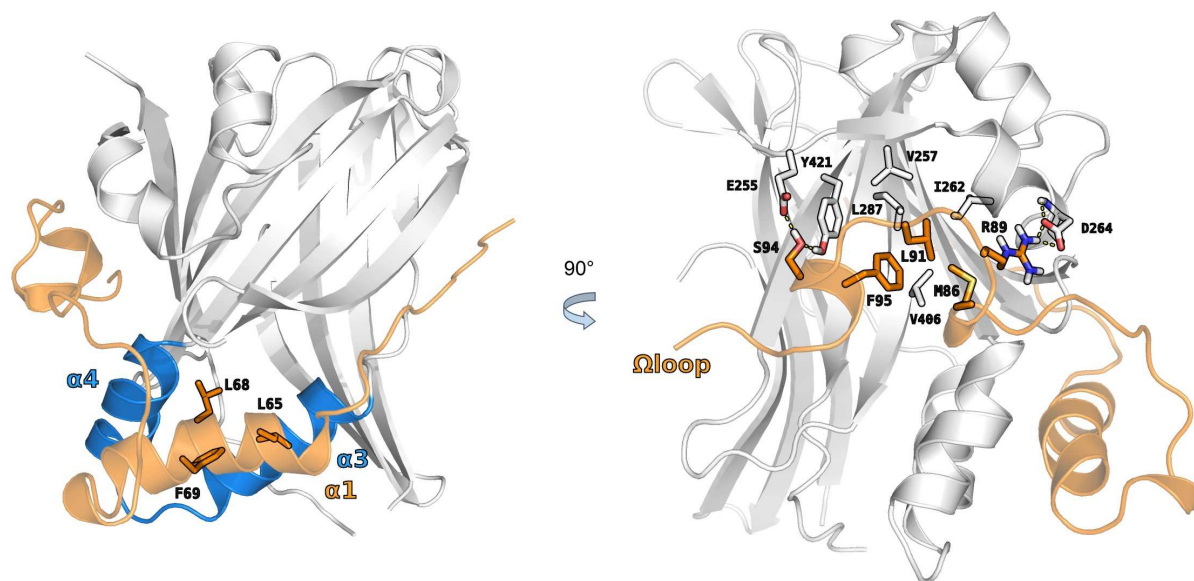


Figure 5:

Figure 6:

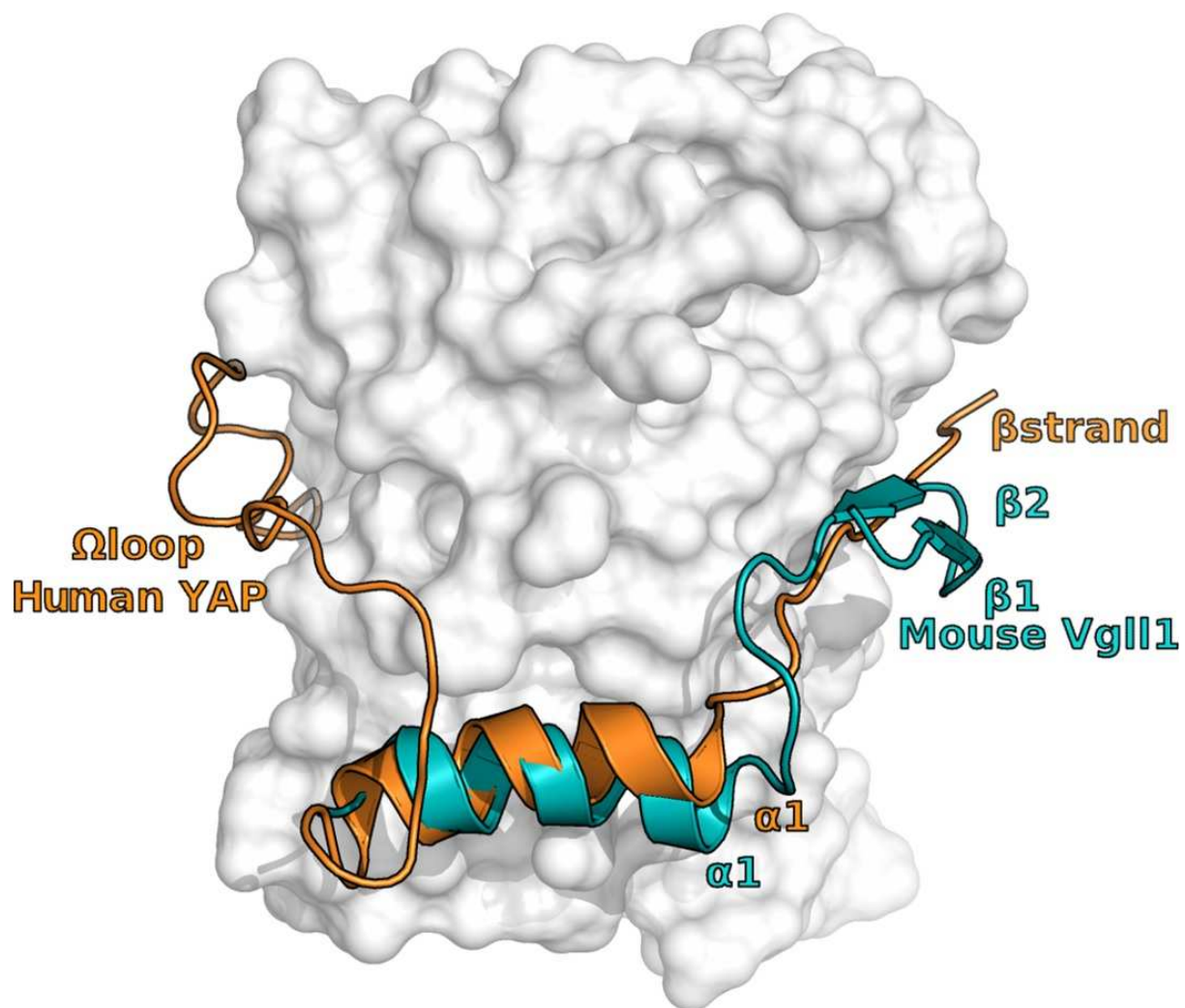
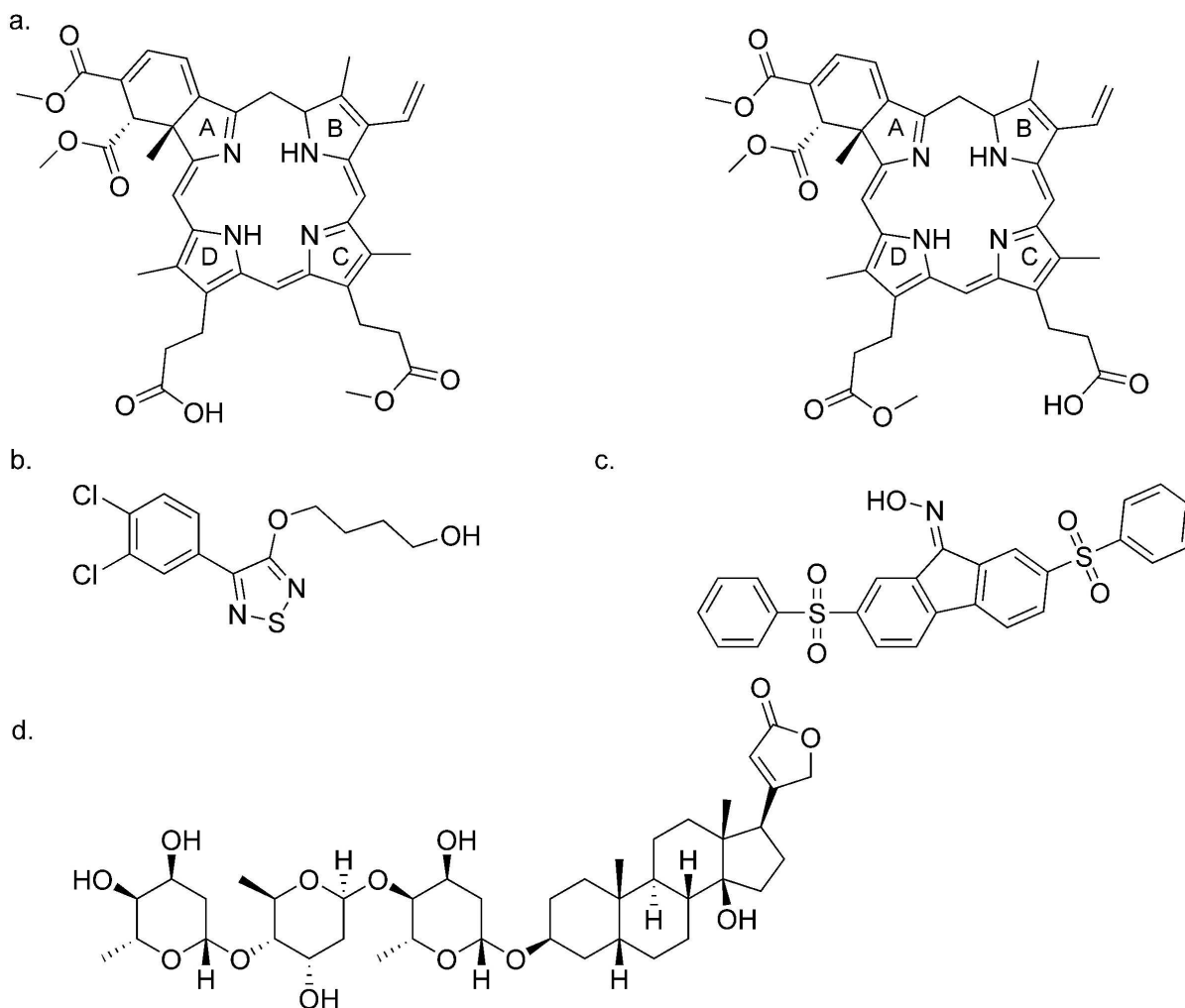


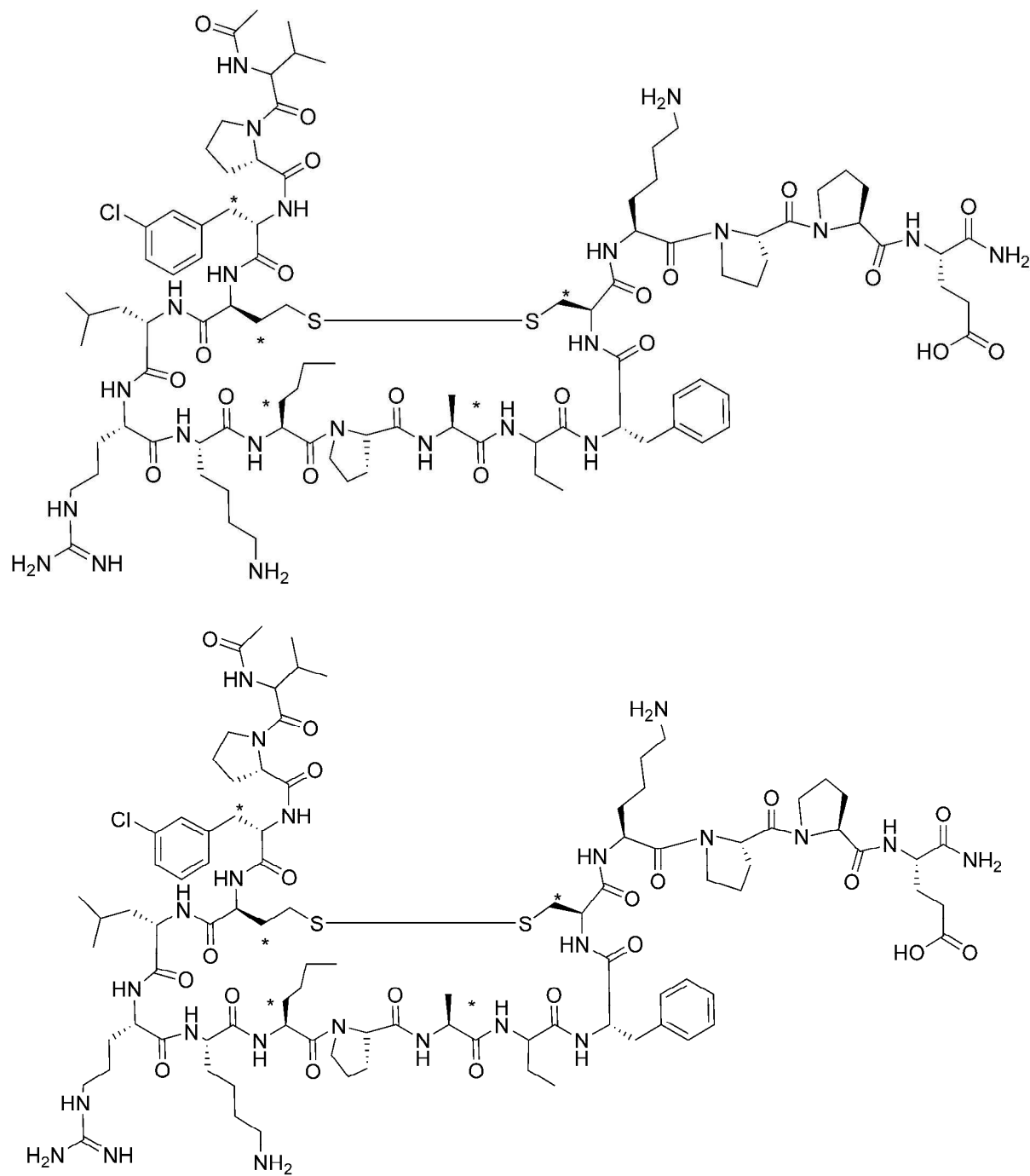


Figure 7:



Figure

8:



**Biographical sketch of each author.**

Matteo Santucci received his master degree in Pharmaceutical Biotechnologies at the University of Modena and Reggio in 2012. He is currently a student of the PhD course “Science and Technologies of Health Products” at the University of Modena, in the laboratory of Prof. Maria Paola Costi. His main research interests concern different protein-enzyme purification; drug-target interaction studies between small peptides/bioactive compounds and target enzyme and enzymatic studies in medium-high throughput screening in drug-preclinical development, including optical, fluorimetric and calorimetric techniques (UV-Vis and fluorescence spectroscopy, isothermal titration calorimetry).

Tatiana Vignudelli studied Medical Biotechnologies at the University of Modena and Reggio Emilia, where she graduated in 2002 after a visiting fellowship at Thomas Jefferson University in Philadelphia (USA). In 2008 she received her PhD in Biotechnology and Molecular Medicine at the University of Modena and Reggio Emilia and she is currently collaborating with Prof. Costi's Lab. Her research has mainly been focused on the molecular mechanisms underlying signal transduction pathways regulating differentiation and proliferation of hematopoietic stem cells.

Stefania Ferrari graduated in Pharmaceutical Chemistry and Technology in 1998 and received her doctorate in the Sciences of Drugs in 2002 at the University of Modena and Reggio Emilia. Since 2002 she has been collaborating with Prof. Costi in many projects focused in design and synthesis of new compounds, computation and biophysical studies of drug-ligand or protein-protein interactions, proteomics and translational research. From 2014 she holds a three-years temporary researcher position at University of Modena and Reggio Emilia.

Marco Mor received his Laurea in Pharmaceutical Chemistry and Technology at the University of Parma (Italy) in 1990. He had collaborated with the Chemoinformatics and Drug Design group at Glaxo Research Center in Verona for three years. In 1993 he became Lecturer at the University of Parma, where he is now Full Professor of Medicinal Chemistry and coordinator of the PhD course in Drugs, Biomolecules and Health Products, at the University of Parma. His research is mainly focused on computer-aided design and SAR analysis of GPCR ligands, tyrosine kinase inhibitors and modulators of the endocannabinoid system.

Laura Scalvini received her master's degree in Pharmaceutical Chemistry and Technology from the University of Parma in 2012. She is currently a student of the PhD course “Design and Synthesis of Biologically Active Compounds” at the University of Parma. Her main research interest is computer-aided design of modulators of endocannabinoid system and small molecules for the modulation of stem cell fate.

Maria Laura Bolognesi received her PhD in Pharmaceutical Sciences in 1996, studying under Carlo Melchiorre at Bologna University. After postdoctoral studies at the University of Minnesota with Philip S. Portoghese, she returned to Bologna University in 1998 as an Assistant Professor and became an Associate Professor in 2005. In 2009 she was awarded a position of Distinguished Visiting Professor at Universidad Complutense de Madrid and in 2014 a position of Special Visiting Researcher (Pesquisador Visitante Especial ) at University of Brasilia. Her research focuses on the design and synthesis of small molecules as probes for the investigation of biological processes or as drug candidates

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2  
3 Elisa Uliassi received her master's degree in Pharmaceutical Chemistry and Technology in 2012  
4 from the University of Bologna. She is currently a doctoral student in the Bolognesi Lab. Her  
5 research work focuses on the development of small molecules for stem cell fate modulation.  
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7  
8 Maria Paola Costi received a degree in Chemistry and Pharmaceutical Science, and in Pharmacy at the  
9 University of Modena. She obtained her PhD in Medicinal Chemistry in Pharmaceutical Science in 1989.  
10 She is professor in Medicinal Chemistry and her expertise is in medicinal chemistry and translational  
11 research. She is actively working in three main research area on the identification and synthesis of leads in  
12 the topic of Thymidylate synthase enzymes structure, function, inhibition and network-pathways in cancer;  
13 folate related enzymes involved in parasitic disease and beta-lactamase structure, function and inhibition.  
14 She has coordinated international projects in the area of ovarian cancer and in infectious diseases.  
15  
16  
17  
18  
19  
20  
21

## 22 REFERENCES:

- 23 [1] Halder G, Johnson RL. Hippo signaling: growth control and beyond. *Development*.  
24 2011;138(1):9-22.  
25  
26 [2] Staley BK, Irvine KD. Hippo signaling in *Drosophila*: recent advances and insights.  
27 *Developmental dynamics : an official publication of the American Association of*  
28 *Anatomists*. 2012;241(1):3-15.  
29  
30 [3] Tremblay AM, Camargo FD. Hippo signaling in mammalian stem cells. *Seminars in cell*  
31 *& developmental biology*. 2012;23(7):818-26.  
32  
33 [4] Zhao B, Li L, Lei Q, Guan KL. The Hippo-YAP pathway in organ size control and  
34 tumorigenesis: an updated version. *Genes & development*. 2010;24(9):862-74.  
35  
36 [5] Zhao B, Tumaneng K, Guan KL. The Hippo pathway in organ size control, tissue  
37 regeneration and stem cell self-renewal. *Nature cell biology*. 2011;13(8):877-83.  
38  
39 [6] Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, et al. Role of  
40 YAP/TAZ in mechanotransduction. *Nature*. 2011;474(7350):179-83.  
41  
42 [7] Yu FX, Guan KL. The Hippo pathway: regulators and regulations. *Genes & development*.  
43 2013;27(4):355-71.  
44  
45 [8] Hong W, Guan KL. The YAP and TAZ transcription co-activators: key downstream  
46 effectors of the mammalian Hippo pathway. *Seminars in cell & developmental biology*.  
47 2012;23(7):785-93.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 [9] Yin M, Zhang L. Hippo signaling: a hub of growth control, tumor suppression and  
4 pluripotency maintenance. *Journal of genetics and genomics = Yi chuan xue bao.*  
5 2011;38(10):471-81.  
6  
7  
8  
9 [10] Ma Y, Yang Y, Wang F, Wei Q, Qin H. Hippo-YAP signaling pathway: A new paradigm  
10 for cancer therapy. *International journal of cancer Journal international du cancer.* 2014.  
11  
12  
13 [11] Mo JS, Park HW, Guan KL. The Hippo signaling pathway in stem cell biology and cancer.  
14 *EMBO reports.* 2014;15(6):642-56.  
15  
16  
17 [12] Pan D. The hippo signaling pathway in development and cancer. *Developmental cell.*  
18 2010;19(4):491-505.  
19  
20  
21 [13] Li Z, Zhao B, Wang P, Chen F, Dong Z, Yang H, et al. Structural insights into the YAP  
22 and TEAD complex. *Genes & development.* 2010;24(3):235-40.  
23  
24  
25 [14] Hiemer SE, Varelas X. Stem cell regulation by the Hippo pathway. *Biochimica et*  
26 *biophysica acta.* 2013;1830(2):2323-34.  
27  
28  
29 [15] Johnson R, Halder G. The two faces of Hippo: targeting the Hippo pathway for  
30 regenerative medicine and cancer treatment. *Nature reviews Drug discovery.*  
31 2014;13(1):63-79.  
32  
33  
34 [16] Ni L, Li S, Yu J, Min J, Brautigam CA, Tomchick DR, et al. Structural basis for  
35 autoactivation of human Mst2 kinase and its regulation by RASSF5. *Structure (London,*  
36 *England : 1993).* 2013;21(10):1757-68.  
37  
38  
39 [17] Praskova M, Khoklatchev A, Ortiz-Vega S, Avruch J. Regulation of the MST1 kinase by  
40 autophosphorylation, by the growth inhibitory proteins, RASSF1 and NORE1, and by Ras.  
41 *The Biochemical journal.* 2004;381(Pt 2):453-62.  
42  
43  
44 [18] Zhao B, Li L, Tumaneng K, Wang CY, Guan KL. A coordinated phosphorylation by Lats  
45 and CK1 regulates YAP stability through SCF(beta-TRCP). *Genes & development.*  
46 2010;24(1):72-85.  
47  
48  
49 [19] Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, et al. Inactivation of YAP oncoprotein  
50 by the Hippo pathway is involved in cell contact inhibition and tissue growth control.  
51 *Genes & development.* 2007;21(21):2747-61.  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 [20] Ribeiro PS, Josue F, Wepf A, Wehr MC, Rinner O, Kelly G, et al. Combined functional  
4 genomic and proteomic approaches identify a PP2A complex as a negative regulator of  
5 Hippo signaling. *Molecular cell*. 2010;39(4):521-34.  
6  
7  
8  
9 [21] Grusche FA, Richardson HE, Harvey KF. Upstream regulation of the hippo size control  
10 pathway. *Current biology : CB*. 2010;20(13):R574-82.  
11  
12  
13 [22] Ikeda M, Kawata A, Nishikawa M, Tateishi Y, Yamaguchi M, Nakagawa K, et al. Hippo  
14 pathway-dependent and -independent roles of RASSF6. *Science signaling*.  
15 2009;2(90):ra59.  
16  
17  
18  
19 [23] Das Thakur M, Feng Y, Jagannathan R, Seppa MJ, Skeath JB, Longmore GD. Ajuba LIM  
20 proteins are negative regulators of the Hippo signaling pathway. *Current biology : CB*.  
21 2010;20(7):657-62.  
22  
23  
24  
25 [24] Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, et al. Elucidation of a  
26 universal size-control mechanism in *Drosophila* and mammals. *Cell*. 2007;130(6):1120-  
27 33.  
28  
29  
30  
31 [25] Neto-Silva RM, de Beco S, Johnston LA. Evidence for a growth-stabilizing regulatory  
32 feedback mechanism between Myc and Yorkie, the *Drosophila* homolog of Yap.  
33 *Developmental cell*. 2010;19(4):507-20.  
34  
35  
36  
37 [26] Li C, Srivastava RK, Elmets CA, Afaq F, Athar M. Arsenic-induced cutaneous  
38 hyperplastic lesions are associated with the dysregulation of Yap, a Hippo signaling-  
39 related protein. *Biochemical and biophysical research communications*. 2013;438(4):607-  
40 12.  
41  
42  
43  
44 [27] Zhang H, Pasolli HA, Fuchs E. Yes-associated protein (YAP) transcriptional coactivator  
45 functions in balancing growth and differentiation in skin. *Proceedings of the National*  
46 *Academy of Sciences of the United States of America*. 2011;108(6):2270-5.  
47  
48  
49  
50 [28] Lai D, Ho KC, Hao Y, Yang X. Taxol resistance in breast cancer cells is mediated by the  
51 hippo pathway component TAZ and its downstream transcriptional targets Cyr61 and  
52 CTGF. *Cancer research*. 2011;71(7):2728-38.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 [29] Liu-Chittenden Y, Huang B, Shim JS, Chen Q, Lee SJ, Anders RA, et al. Genetic and  
4 pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity  
5 of YAP. *Genes & development*. 2012;26(12):1300-5.  
6  
7  
8  
9 [30] Fossdal R, Jonasson F, Kristjansdottir GT, Kong A, Stefansson H, Gosh S, et al. A novel  
10 TEAD1 mutation is the causative allele in Sveinsson's chorioretinal atrophy (helicoid  
11 peripapillary chorioretinal degeneration). *Human molecular genetics*. 2004;13(9):975-81.  
12  
13  
14 [31] Zhao B, Ye X, Yu J, Li L, Li W, Li S, et al. TEAD mediates YAP-dependent gene  
15 induction and growth control. *Genes & development*. 2008;22(14):1962-71.  
16  
17  
18  
19 [32] Murakami M, Nakagawa M, Olson EN, Nakagawa O. A WW domain protein TAZ is a  
20 critical coactivator for TBX5, a transcription factor implicated in Holt-Oram syndrome.  
21 *Proceedings of the National Academy of Sciences of the United States of America*.  
22 2005;102(50):18034-9.  
23  
24  
25  
26 [33] Murakami M, Tominaga J, Makita R, Uchijima Y, Kurihara Y, Nakagawa O, et al.  
27 Transcriptional activity of Pax3 is co-activated by TAZ. *Biochemical and biophysical  
28 research communications*. 2006;339(2):533-9.  
29  
30  
31  
32 [34] Park KS, Whitsett JA, Di Palma T, Hong JH, Yaffe MB, Zannini M. TAZ interacts with  
33 TTF-1 and regulates expression of surfactant protein-C. *The Journal of biological  
34 chemistry*. 2004;279(17):17384-90.  
35  
36  
37  
38 [35] Di Palma T, D'Andrea B, Liguori GL, Liguoro A, de Cristofaro T, Del Prete D, et al. TAZ  
39 is a coactivator for Pax8 and TTF-1, two transcription factors involved in thyroid  
40 differentiation. *Experimental cell research*. 2009;315(2):162-75.  
41  
42  
43  
44 [36] Alarcon C, Zaromytidou AI, Xi Q, Gao S, Yu J, Fujisawa S, et al. Nuclear CDKs drive  
45 Smad transcriptional activation and turnover in BMP and TGF-beta pathways. *Cell*.  
46 2009;139(4):757-69.  
47  
48  
49  
50 [37] Varelas X, Sakuma R, Samavarchi-Tehrani P, Peerani R, Rao BM, Dembowy J, et al. TAZ  
51 controls Smad nucleocytoplasmic shuttling and regulates human embryonic stem-cell self-  
52 renewal. *Nature cell biology*. 2008;10(7):837-48.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 [38] Strano S, Munarriz E, Rossi M, Castagnoli L, Shaul Y, Sacchi A, et al. Physical  
4 interaction with Yes-associated protein enhances p73 transcriptional activity. *The Journal*  
5 *of biological chemistry*. 2001;276(18):15164-73.  
6  
7  
8  
9 [39] Zaidi SK, Sullivan AJ, Medina R, Ito Y, van Wijnen AJ, Stein JL, et al. Tyrosine  
10 phosphorylation controls Runx2-mediated subnuclear targeting of YAP to repress  
11 transcription. *The EMBO journal*. 2004;23(4):790-9.  
12  
13  
14 [40] Westendorf JJ. Transcriptional co-repressors of Runx2. *Journal of cellular biochemistry*.  
15 2006;98(1):54-64.  
16  
17  
18 [41] Komuro A, Nagai M, Navin NE, Sudol M. WW domain-containing protein YAP  
19 associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal  
20 fragment of ErbB-4 that translocates to the nucleus. *The Journal of biological chemistry*.  
21 2003;278(35):33334-41.  
22  
23  
24 [42] Zhao B, Li L, Lu Q, Wang LH, Liu CY, Lei Q, et al. Angiomotin is a novel Hippo  
25 pathway component that inhibits YAP oncoprotein. *Genes & development*. 2011;25(1):51-  
26 63.  
27  
28  
29 [43] Hirate Y, Sasaki H. The role of angiomotin phosphorylation in the Hippo pathway during  
30 preimplantation mouse development. *Tissue barriers*. 2014;2(1):e28127.  
31  
32  
33 [44] Mana-Capelli S, Paramasivam M, Dutta S, McCollum D. Angiomotins link F-actin  
34 architecture to Hippo pathway signaling. *Molecular biology of the cell*. 2014;25(10):1676-  
35 85.  
36  
37  
38 [45] Hong W. Angiomotin's YAP into the nucleus for cell proliferation and cancer  
39 development. *Science signaling*. 2013;6(291):pe27.  
40  
41  
42 [46] Oka T, Remue E, Meerschaert K, Vanloo B, Boucherie C, Gfeller D, et al. Functional  
43 complexes between YAP2 and ZO-2 are PDZ domain-dependent, and regulate YAP2  
44 nuclear localization and signaling. *The Biochemical journal*. 2010;432(3):461-72.  
45  
46  
47 [47] Oka T, Schmitt AP, Sudol M. Opposing roles of angiomotin-like-1 and zona occludens-2  
48 on pro-apoptotic function of YAP. *Oncogene*. 2012;31(1):128-34.  
49  
50  
51 [48] Schlegelmilch K, Mohseni M, Kirak O, Pruszkak J, Rodriguez JR, Zhou D, et al. Yap1 acts  
52 downstream of alpha-catenin to control epidermal proliferation. *Cell*. 2011;144(5):782-95.  
53  
54  
55  
56  
57  
58  
59  
60



- 1  
2  
3 [49] Silvis MR, Kreger BT, Lien WH, Klezovitch O, Rudakova GM, Camargo FD, et al. alpha-  
4 catenin is a tumor suppressor that controls cell accumulation by regulating the localization  
5 and activity of the transcriptional coactivator Yap1. *Science signaling*. 2011;4(174):ra33.  
6  
7  
8  
9 [50] Varelas X, Samavarchi-Tehrani P, Narimatsu M, Weiss A, Cockburn K, Larsen BG, et al.  
10 The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-  
11 beta-SMAD pathway. *Developmental cell*. 2010;19(6):831-44.  
12  
13  
14 [51] Ramos A, Camargo FD. The Hippo signaling pathway and stem cell biology. *Trends in*  
15 *cell biology*. 2012;22(7):339-46.  
16  
17  
18  
19 [52] Yagi R, Kohn MJ, Karavanova I, Kaneko KJ, Vullhorst D, DePamphilis ML, et al.  
20 Transcription factor TEAD4 specifies the trophectoderm lineage at the beginning of  
21 mammalian development. *Development*. 2007;134(21):3827-36.  
22  
23  
24 [53] Nishioka N, Yamamoto S, Kiyonari H, Sato H, Sawada A, Ota M, et al. Tead4 is required  
25 for specification of trophectoderm in pre-implantation mouse embryos. *Mechanisms of*  
26 *development*. 2008;125(3-4):270-83.  
27  
28  
29  
30 [54] Nishioka N, Inoue K, Adachi K, Kiyonari H, Ota M, Ralston A, et al. The Hippo signaling  
31 pathway components Lats and Yap pattern Tead4 activity to distinguish mouse  
32 trophectoderm from inner cell mass. *Developmental cell*. 2009;16(3):398-410.  
33  
34  
35 [55] Lian I, Kim J, Okazawa H, Zhao J, Zhao B, Yu J, et al. The role of YAP transcription  
36 coactivator in regulating stem cell self-renewal and differentiation. *Genes & development*.  
37 2010;24(11):1106-18.  
38  
39  
40 [56] Barry ER, Camargo FD. The Hippo superhighway: signaling crossroads converging on the  
41 Hippo/Yap pathway in stem cells and development. *Current opinion in cell biology*.  
42 2013;25(2):247-53.  
43  
44  
45 [57] Widelitz RB. Wnt signaling in skin organogenesis. *Organogenesis*. 2008;4(2):123-33.  
46  
47  
48  
49 [58] Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R, et al. YAP1 increases  
50 organ size and expands undifferentiated progenitor cells. *Current biology : CB*.  
51 2007;17(23):2054-60.  
52  
53  
54 [59] Zhou D, Zhang Y, Wu H, Barry E, Yin Y, Lawrence E, et al. Mst1 and Mst2 protein  
55 kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of  
56  
57  
58  
59  
60

1  
2  
3 Yes-associated protein (Yap) overabundance. Proceedings of the National Academy of  
4 Sciences of the United States of America. 2011;108(49):E1312-20.  
5  
6

7 [60] Gee ST, Milgram SL, Kramer KL, Conlon FL, Moody SA. Yes-associated protein 65  
8 (YAP) expands neural progenitors and regulates Pax3 expression in the neural plate border  
9 zone. PloS one. 2011;6(6):e20309.  
10  
11

12 [61] Fernandez LA, Northcott PA, Dalton J, Fraga C, Ellison D, Angers S, et al. YAP1 is  
13 amplified and up-regulated in hedgehog-associated medulloblastomas and mediates Sonic  
14 hedgehog-driven neural precursor proliferation. Genes & development. 2009;23(23):2729-  
15 41.  
16  
17  
18

19 [62] Lee KP, Lee JH, Kim TS, Kim TH, Park HD, Byun JS, et al. The Hippo-Salvador pathway  
20 restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. Proceedings of  
21 the National Academy of Sciences of the United States of America. 2010;107(18):8248-  
22 53.  
23  
24  
25  
26  
27

28 [63] Song H, Mak KK, Topol L, Yun K, Hu J, Garrett L, et al. Mammalian Mst1 and Mst2  
29 kinases play essential roles in organ size control and tumor suppression. Proceedings of  
30 the National Academy of Sciences of the United States of America. 2010;107(4):1431-6.  
31  
32  
33

34 [64] Lu L, Li Y, Kim SM, Bossuyt W, Liu P, Qiu Q, et al. Hippo signaling is a potent in vivo  
35 growth and tumor suppressor pathway in the mammalian liver. Proceedings of the  
36 National Academy of Sciences of the United States of America. 2010;107(4):1437-42.  
37  
38  
39

40 [65] Heallen T, Zhang M, Wang J, Bonilla-Claudio M, Klysik E, Johnson RL, et al. Hippo  
41 pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size.  
42 Science (New York, NY). 2011;332(6028):458-61.  
43  
44  
45

46 [66] Xin M, Kim Y, Sutherland LB, Qi X, McAnally J, Schwartz RJ, et al. Regulation of  
47 insulin-like growth factor signaling by Yap governs cardiomyocyte proliferation and  
48 embryonic heart size. Science signaling. 2011;4(196):ra70.  
49  
50  
51

52 [67] Sudol M, Shields DC, Farooq A. Structures of YAP protein domains reveal promising  
53 targets for development of new cancer drugs. Seminars in cell & developmental biology.  
54 2012;23(7):827-33.  
55  
56  
57  
58  
59  
60

- 1  
2  
3 [68] Kanai F, Marignani PA, Sarbassova D, Yagi R, Hall RA, Donowitz M, et al. TAZ: a novel  
4 transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain  
5 proteins. *The EMBO journal*. 2000;19(24):6778-91.  
6  
7  
8  
9 [69] Tian W, Yu J, Tomchick DR, Pan D, Luo X. Structural and functional analysis of the  
10 YAP-binding domain of human TEAD2. *Proceedings of the National Academy of*  
11 *Sciences of the United States of America*. 2010;107(16):7293-8.  
12  
13  
14 [70] Hau JC, Erdmann D, Mesrouze Y, Furet P, Fontana P, Zimmermann C, et al. The TEAD4-  
15 YAP/TAZ protein-protein interaction: expected similarities and unexpected differences.  
16 *Chembiochem : a European journal of chemical biology*. 2013;14(10):1218-25.  
17  
18  
19  
20 [71] Anbanandam A, Albarado DC, Nguyen CT, Halder G, Gao X, Veeraraghavan S. Insights  
21 into transcription enhancer factor 1 (TEF-1) activity from the solution structure of the  
22 TEA domain. *Proceedings of the National Academy of Sciences*. 2006;103(46):17225-30.  
23  
24  
25  
26 [72] Chen L, Chan SW, Zhang X, Walsh M, Lim CJ, Hong W, et al. Structural basis of YAP  
27 recognition by TEAD4 in the hippo pathway. *Genes & development*. 2010;24(3):290-300.  
28  
29  
30  
31 [73] Pobbati AV, Chan SW, Lee I, Song H, Hong W. Structural and functional similarity  
32 between the Vgll1-TEAD and the YAP-TEAD complexes. *Structure (London, England :*  
33 *1993)*. 2012;20(7):1135-40.  
34  
35  
36  
37 [74] Mesrouze Y, Hau JC, Erdmann D, Zimmermann C, Fontana P, Schmelzle T, et al. The  
38 surprising features of the TEAD4-Vgll1 protein-protein interaction. *Chembiochem : a*  
39 *European journal of chemical biology*. 2014;15(4):537-42.  
40  
41  
42  
43 [75] Jiao S, Wang H, Shi Z, Dong A, Zhang W, Song X, et al. A peptide mimicking VGLL4  
44 function acts as a YAP antagonist therapy against gastric cancer. *Cancer cell*.  
45 2014;25(2):166-80.  
46  
47  
48  
49 [76] Casey FP, Pihan E, Shields DC. Discovery of small molecule inhibitors of protein-protein  
50 interactions using combined ligand and target score normalization. *Journal of chemical*  
51 *information and modeling*. 2009;49(12):2708-17.  
52  
53  
54  
55 [77] Yu FX, Zhao B, Panupinthu N, Jewell JL, Lian I, Wang LH, et al. Regulation of the  
56 Hippo-YAP pathway by G-protein-coupled receptor signaling. *Cell*. 2012;150(4):780-91.  
57  
58  
59  
60

- 1  
2  
3 [78] Miller E, Yang J, DeRan M, Wu C, Su AI, Bonamy GM, et al. Identification of serum-  
4 derived sphingosine-1-phosphate as a small molecule regulator of YAP. *Chemistry &*  
5 *biology*. 2012;19(8):955-62.  
6  
7  
8  
9 [79] Guan KL, YU F, Ding S. Inhibitors of hippo-yap signaling pathway. World Intellectual  
10 Property Organization WO 2013/188138 A1, December 19, 2013.  
11  
12  
13 [80] Brodowska K, Al-Moujahed A, Marmalidou A, Meyer Zu Horste M, Cichy J, Miller JW,  
14 et al. The clinically used photosensitizer Verteporfin (VP) inhibits YAP-TEAD and human  
15 retinoblastoma cell growth in vitro without light activation. *Experimental eye research*.  
16 2014;124:67-73.  
17  
18  
19  
20 [81] Zhang Z, Lin Z, Zhou Z, Shen HC, Yan SF, Mayweg AV, et al. Structure-Based Design  
21 and Synthesis of Potent Cyclic Peptides Inhibiting the YAP/TEAD Protein-Protein  
22 Interaction. *ACS Medicinal Chemistry Letters*. 2014;5(9):993-8.  
23  
24  
25  
26 [82] Basu D, Lettan R, Damodaran K, Strellec S, Reyes-Mugica M, Rebbaa A. Identification,  
27 mechanism of action, and antitumor activity of a small molecule inhibitor of hippo, TGF-  
28 beta, and Wnt signaling pathways. *Molecular cancer therapeutics*. 2014;13(6):1457-67.  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
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