The Hippo pathway regulates organ size by controlling both cell proliferation and apoptosis. TAZ functions as a transcriptional co-activator downstream of the Hippo pathway and has been implicated in human cancer development. A key step in the Hippo-TAZ pathway is phosphorylation of TAZ by LATS kinase, which leads to TAZ inhibition by both cytoplasmic retention and degradation. However, the mechanism of TAZ dephosphorylation and the responsible phosphatase are unknown. Here we identified PP1 as a bona fide TAZ phosphatase. PP1A dephosphorylates TAZ at Ser89 and Ser311, promotes TAZ nuclear translocation, and stabilizes TAZ by disrupting the binding to the SCF E3 ubiquitin ligase. Furthermore, ASPP2 facilitates the interaction between TAZ and PP1 to promote TAZ dephosphorylation. As a result, PP1 and ASPP2 increase TAZ dependent gene expression. This study demonstrates that PP1A and ASPP2 play a critical role in promoting TAZ function by antagonizing the Lats kinase through TAZ dephosphorylation.

Genetic screens in Drosophila have delineated a new tumor suppressor pathway, the Hippo pathway that regulates organ size by controlling both cell proliferation and apoptosis(1). Both the components and functions of the Hippo pathway are conserved from Drosophila to mammals. In mammalian cells, LATS1/2 and MST1/2 are the homologues of Drosophila Warts and Hippo, respectively (2). YAP, the mammalian ortholog of Drosophila Yorkie, has been demonstrated to be phosphorylated and inhibited by LATS (3,4). TAZ, first identified as a 14-3-3 binding protein, shares approximately 50% sequence identity with YAP and has also been shown to function as a transcriptional co-activator downstream of the Hippo pathway (5,6). TAZ is involved in the development of multiple organs such as lung, fat, muscle, bone, limb, and heart as well as many cellular processes including stem cell differentiation, cell proliferation, epithelial mesenchymal transition (EMT) (6-12). Taz knockout mice develop two severe abnormalities: polycystic kidney disease and emphysema (13,14). Notably, elevated TAZ expression is observed in more than 20% of breast cancers, especially invasive ductal carcinomas (12).

We previously showed that the activity of TAZ is inhibited by LATS kinase in a mechanism similar to YAP regulation by the Hippo pathway (6). There are four HXRXXS LATS phosphorylation motifs in TAZ. We have demonstrated that phosphorylation of TAZ at S89 by LATS promotes its interaction with 14-3-3, resulting in cytoplasmic sequestration and
functional inhibition of TAZ. S89A mutation confers TAZ partially resistant to inhibition by the Hippo pathway (6). In addition, TAZ stability is also regulated by LATS phosphorylation. A dual phosphorylation of TAZ by LATS and CK1, which creates a binding site for β-TrCP, the substrate recruiting subunit of the SCFβ-TrCP E3 ubiquitin ligase(15). Phosphorylation of TAZ at Ser311 by LATS provides the priming site for subsequent CK1 phosphorylation at Ser314 in TAZ, therefore creates a functional phosphodegron for β-TrCP binding (15). Therefore, phosphorylation plays a major role in TAZ regulation in both subcellular localization and protein levels.

Protein phosphorylation is highly dynamic and reversible. This raises questions whether and which protein phosphatases are involve in TAZ regulation. Protein Ser/Thr phosphatases are classified into three families: PPM, PPP, and FCP/SCP (16,17). The PPP superfamily members, including PP1, PP2A (PP2), PP2B (PP3) and PP4-7, share the same catalytic mechanism(18). PP1 (Protein phosphatase1) is a major eukaryotic protein Ser/Thr phosphatase involved in various cellular processes (19). Interestingly, in our previous TAZ affinity purification to identify TAZ binding proteins (20), we also identified PP1A (a catalytic subunit of PP1) in TAZ complex, indicating PP1A is potentially TAZ phosphatase. Notably, PP1A has been reported to interact with ASPP2(21), which is a member of the ASPP (ankyrin-repeat-containing, SH3-domain-containing, and proline-rich-region-containing protein) family, containing four ankyrin repeats, an SH3 domain, and a proline rich region(22-24). Through C-terminal ankyrin repeats and SH3 domain, ASPP2 has been shown to interact with numerous proteins, including p53 (25), Bcl-2 (26), RelA p65(27), APC-2(APCL) (28), Yap (29), and APP-BP1 (30). YAP, mammalian homolog of TAZ, interacts with ASPP2 through WW domain and SH3-domain binding motif, but the biochemical and biological significance underlying the YAP-ASPP2 interaction is largely unknown.

In this report, we showed that PP1A dephosphorylates TAZ at Ser89 and Ser311. By doing so, PP1A increases TAZ nuclear localization and protein levels. We also observed that ASPP2 facilitates the interaction between TAZ and PP1A, thus leads to TAZ dephosphorylation and induction of TAZ target gene expression. Our study identifies PP1A as a TAZ phosphatase and the function of ASPP2 and PP1A in TAZ regulation.

EXPERIMENTAL PROCEDURES

Cell culture and transfection.
HEK293T cells and HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum (HyClone), 100 U/ml penicillin, and streptomycin (Gibco). MCF10A cells were maintained in DMEM/F-12 medium (Invitrogen) supplemented with 5% horse serum (Invitrogen), 20 ng/ml epidermal growth factor, 0.5μg/ml hydrocortisone, 10 μg/ml insulin, 100ng/ml cholera toxin, 100U/ml penicillin, and streptomycin (Gibco). Cell transfection was performed using Lipofectamine 2000 (Invitrogen) or calcium phosphate method. Cells were harvested at 24 h post transfection for protein analyses.

To establish stable TAZ-expressing and PP1α-expressing cells, pBabe-TAZ and pQCXIH-PP1α retroviruses were generated and used to infect MCF10A cells and stable pools were selected in puromycin (1μg/ml) and hygromycin (200μg/ml) respectively.

Western blotting analysis and immunoprecipitation.
Protein lysates were prepared by lysing 293T cells in a buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1% SDS, 0.5% deoxycholate, 1% NP-40, 1 mM EDTA, 1 mM PMSF, 25 mM NaF, and cocktail protease inhibitors (Roche).
Cell lysate (40 μg) was resolved by SDS-PAGE, followed by Western blotting analysis. Antibodies of Flag (A00170 from GenScript or A2220 from Sigma), HA(F7) (Santa Cruz, SC7392), His (THERMIS™ His Tag Antibody, GenScript), ASPP2 (611354, BD), Lamin A+C (A01455, GenScript), α-tubulin (RB-9281-P0, Labvision) and β-actin (13E5)(4970, Cell Signaling) were purchased commercially. Phospho-Ser311 and Phospho-Ser89 TAZ antibodies were raised in rabbits using synthetic phosphopeptide as an antigen.

For immunoprecipitation experiments, 1mg of cell lysate was incubated with anti-Flag M2-agarose for 3h at 4°C. Beads were washed three times with lysis buffer and centrifuged at 5,000g for 5 min between each wash. Protein was eluted from beads with 50 μl of Laemmli sample buffer (Bio-Rad). Lysates were resolved on 8% or 10% SDS-PAGE gels and transferred onto nitrocellulose (Bio-Rad) for Western blotting.

Phosphatase assay
Protein Phosphatase 1 (P0754S) was purchased from NEB. 293T cells were co-transfected with Flag-TAZ and HA-LATS2. After 24 hours incubation, 293T cells were lysed with lysis buffer and the cell lysate was incubated with anti-Flag M2-agarose for 3h at 4°C. Beads were washed three times with lysis buffer first and then with 1X NEBuffer for PMP supplemented with MnCl2. The Flag-TAZ was eluted by 3X Flag peptide in 1X NEBuffer for PMP supplemented with MnCl2. The elution was divided equally and added with indicated amount of PP1. After incubation 30 min at 30°C, the eluted lysates were added with SDS loading buffer and boiled at 99°C. The phosphorylation level of Flag-TAZ was determined by Western blotting.

Subcellular fractionation
293T cells was washed with PBS and transferred to a 1.5ml tube. The cells were lysed with Harvest buffer (10mM HEPES pH 7.9, 50mM NaCl, 0.5M Sucrose, 0.1mM EDTA, 0.5% Triton X 100, 1mM DTT, 1 mM PMSF, 25 mM NaF, and cocktail protease inhibitors ). After incubation 5 min on ice, the lysate was centrifuged at 1000rpm for 10 min. The pellet (nuclear fraction) was washed three times with Wash buffer (10mM HEPES pH 7.9, 10mM KCl, 0.1 EDTA, 0.1 EGTA) and then resuspended with 1 X SDS loading buffer. The supernatant (cytoplasmic fraction) was transferred to a new tube and centrifuged at 14000rpm for another 15min. The supernatant was transferred to a new tube and boiled in 1 X SDS loading buffer.

RNA isolation and real-time PCR.
Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen). cDNA was synthesized by reverse transcription using oligo(dT) as the primer and proceeded to real-time PCR with gene-specific primers in the presence of SYBR Premix Ex Taq (DRR041A; TaKaRa). The relative abundance of mRNA was calculated by normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

Results
PP1A is a TAZ phosphatase
As a transcription co-activator, TAZ must interact with a downstream transcription factor in order to induce gene expression. In our previous work, we performed TAZ affinity purification in order to identify TAZ target transcription factors (20). Interestingly, this purification strategy identified multiple putative TAZ interacting proteins, which included not only all four TEAD family members but also PP1A. In fact, multiple peptides for PP1A protein were identified (Figure 1A). These peptides were absent from control purification of cells infected with the SBP vector. To confirm the interaction between TAZ and PP1A, we performed co-immunoprecipitation (co-IP) experiments and found that HA-PP1A could be readily pulled down by TAZ (Figure 1B, left panel). Similarly, reciprocal co-IP experiments also
showed that TAZ was co-precipitated with PP1A (Figure 1B, right panel).

We have recently characterized that the conserved HXRXXS motifs in YAP and TAZ (3,6). Both YAP and TAZ are phosphorylated by LATS kinase(3,6). However, the phosphatase responsible for dephosphorylation of both YAP and TAZ is unknown. The identification of PP1A in the TAZ purification led us to test if PP1A is a phosphatase for TAZ. To this end, we examined the effect of PP1A on TAZ phosphorylation at Ser89. As shown in Figure 1C, ectopic expression of LATS dramatically induced TAZ phosphorylation at Ser89, but co-expression of PP1A significantly decreased TAZ phosphorylation at Ser89 in a dose-dependent manner. PP1A belongs to members of the PPP family, which includes PP1 and PP2A. Recently, a PP2A complex has been reported to function as a negative regulator of Hippo pathway in Drosophila (31). To identify if the above observed dephosphorylation of TAZ is unique to PP1A, we also examined PP2A on TAZ phosphorylation at Ser89. Interestingly, only overexpression of PP1A WT, but not the phosphatase inactive mutant H248K or PP2A, dramatically decreased TAZ phosphorylation at Ser89 (Figure 1D). Furthermore, we performed in vitro dephosphorylation assay and found that TAZ phosphorylation at Ser89 was decreased with the increasing amount of PP1 (Figure 1E).

PP1A is inhibited by okadaic acid with IC50 of 20nM while PP2A is much more sensitive to okadaic acid with IC50 of 0.2nM (32). Calyculin A is a more efficient inhibitor for both PP1A and PP2A(33). Indeed, both okadaic acid and calyculin A significantly increased TAZ phosphorylation at Ser89 and resulted in an obvious TAZ mobility shift as detected by the TAZ antibody, indicating endogenous TAZ is dynamically regulated by reversible phosphorylation (Figure 1F and Supplemental S1). The concentrations of okadaic acid and calyculin A needed to increase TAZ phosphorylation were consistent with PP1A as a physiological TAZ phosphatase.

**PP1A stabilizes TAZ by decreasing the interaction between TAZ and β-TrCP**

Our recent work has shown LATS and CK1 phosphorylate TAZ and promote TAZ interaction with the SCF E3 ubiquitin ligase and degradation(15). If PP1A dephosphorylates TAZ, it should stabilize TAZ by antagonizing the effect of Lats. To this end, we examined the effect of okadaic acid on TAZ protein levels. As shown in Figure 2A, okadaic acid treatment resulted in a visible decrease of TAZ protein level and an increased TAZ phosphorylation. MG132 treatment blocked the effect of okadaic acid on TAZ protein levels, indicating that PP1A increases TAZ protein by suppressing proteasome mediated degradation. In order to further characterize the PP1A effect on TAZ stability, we generated PP1A stable pools using retrovirus. We observed an obvious increase of TAZ protein in the PP1A stably expressing pBAB-FLAG-TAZ MCF10A cells (Figure 2B). Furthermore, the half –life of TAZ in the PP1A expressing cells was significantly increased comparing to that in the vector control cells (Figure 2C).

Our recent study identified β-TrCP as TAZ binding protein to recruit the SCF E3 ubiquitin ligase(15). We then determined the effect of PP1A on the interaction between TAZ and β-TrCP. PP1A overexpression decreased the interaction between TAZ and β-TrCP both in the absence or presence Lats co-expression (Figure 2D). Furthermore, okadaic acid treatment increased TAZ phosphorylation and its interaction with β-TrCP (Figure 2E). TAZ phosphorylation at Ser311 is required for C-terminal phosphodegron mediated degradation(15). We then determined the effect of PP1A on TAZ phosphorylation at Ser311. We observed a dramatically decreased TAZ phosphorylation at Ser311 in the presence of PP1A (Figure 2F). As expected, PP1A efficiently
dephosphorylated TAZ at Ser311 in vitro (Figure 2G). Consistently, both okadaic acid and calyculin A significantly increased TAZ phosphorylation at Ser311 (Figure 2H). These results demonstrate that PP1A stabilizes TAZ by dephosphorylating TAZ at S311 and decreasing its interaction with β-TrCP.

**ASPP2 increases the interaction between TAZ and PP1A to promote TAZ dephosphorylation**

ASPP2 has been reported to interact with multiple proteins including PP1 (21-24). It has also been reported to interact with the TAZ paralog YAP (29). Indeed, ASPP2 was identified as TAZ interacting protein with high score in our TAZ SBP-MS/MS experiment (Figure S2). Therefore, we speculated whether ASPP2 may facilitate the interaction between TAZ and PP1. To explore the functional role of ASPP2 in TAZ dephosphorylation via PP1, we first tested if TAZ and PP1A can interact with ASPP2. Both TAZ and PP1A could readily pull down ASPP2 (Figure 3A). We also found that ASPP2 increased the interaction between TAZ and PP1A (Figure 3B). Furthermore, TAZ WW mutant abolished its interaction with ASPP2 (Figure 3C), similar with previous report that YAP interacts with ASPP2 via its WW1 domain (29). In addition, ASPP2 increased the interaction between WT TAZ and PP1A, but had no promoting effect on TAZ WW mutant interaction with PP1A (Figure 3D). Moreover, ASPP2 overexpression dramatically decreased WT TAZ phosphorylation at Ser89, but less effect on TAZ WW mutants (Figure 3E and S3). The above results suggest that ASPP2 may stimulate TAZ dephosphorylation partly by promoting the interaction between TAZ and PP1 and this function of ASPP2 requires the TAZ WW domain.

It has been reported that a short peptide from ASPP2 containing the PP1-binding motif (RVXF) interacts with the catalytic subunit of PP1_1 (34). We then tested whether the ability to interact with PP1A is required for ASPP2 to promote TAZ dephosphorylation. As shown in Figure 3F, WT ASPP2 overexpression, dramatically decreased TAZ phosphorylation at Ser89, while the PP1 interacting defective RV mutant, in which the arginine and valine in the RVXF motif were replaced by glutamate and aspartate, totally blocked this effect. In contrast, ASPP2 but not scramble RNAi oligos significantly increased TAZ phosphorylation at Ser89 (Fig 3G). These data support a role of endogenous ASSP2 in promoting TAZ dephosphorylation and a potential mechanism by increasing the interaction between TAZ and PP1A.

**PP1A and ASPP2 promote TAZ nuclear localization**

TAZ phosphorylation at S89 creates a 14-3-3 binding site results in cytoplasmic retention. This suggests that TAZ dephosphorylation by PP1A could affect its interaction with 14-3-3 and subcellular localization. To this end, we examined the binding between TAZ and 14-3-3 with or without PP1A co-expression. We observed that PP1A overexpression significantly decreased the binding between TAZ and 14-3-3 (Figure 4A). Consistently, phosphatase inhibitor calyculin A and okadaic acid treatment increased the interaction between TAZ and 14-3-3(Figure 4B). We also observed that co-expression of ASPP2 WT significantly decreased the interaction between TAZ and 14-3-3. In contrast, the RV mutant was totally inactive to affect the binding between TAZ and 14-3-3 (Figure 4C). Furthermore, PP1A overexpression resulted in a dramatic increase of TAZ nuclear localization and ASPP2 had a similar effect (Figure 4D). Moreover, okadaic acid treatment led to TAZ cytoplasmic retention (Figure 4E). Collectively, our data suggest that PP1A and ASPP2 promote TAZ nuclear localization via dephosphorylation.

**PP1A and ASPP2 regulate TAZ target gene expression**

TAZ is known to induce EMT and promote
cell proliferation (6,12). As a transcription co-activator, TAZ exerts its biological function by modulating gene expression. To determine the functional significance of PP1A and ASPP2 in TAZ regulation, we performed qPCR to examine the PP1A and ASPP2 effect on EMT makers and cell growth markers, which are regulated by TAZ. As shown in Figure 5A, PP1A overexpression significantly enhanced the ability of TAZ to induce N-cadherin, a marker for mesenchymal cells, and reduce E-cadherin, a marker for epithelial cells, as determined in MCF10A stably expressing TAZ and PP1A. Similarly, co-expression of PP1A increased the TAZ-induced expression of CTGF, which is a TAZ target gene involved in promoting cell growth (Figure 5A). Furthermore, ASPP2 knockdown significantly decreased CTGF expression in MCF10A stably expressing TAZ but had a much weaker effect in cells stably expressing TAZ<sup>ASPA</sup>, which is a constitutively active mutant no longer inhibited by phosphorylation (Figure 5B). Collectively, the above data support that PP1 and ASPP2 activate TAZ as indicated by the increase of TAZ-dependent gene expression. Moreover, the effect of PP1 and ASPP2 on TAZ target gene expression appears to be mediated via TAZ dephosphorylation.

**Discussion**

In this report, we show that both PP1A and ASPP2 promote TAZ dephosphorylation, therefore resulting in TAZ nuclear retention and induction of TAZ target gene expression. Our study reveals a molecular mechanism of dynamic reversible phosphorylation in regulation of TAZ by the Hippo pathway and PP1A.

We have previously demonstrated that phosphorylation negatively regulates TAZ by two mechanisms. Phosphorylation of Ser89 inhibits TAZ by cytoplasmic sequestration while phosphorylation of Ser311 inactivates TAZ by promoting ubiquitination and degradation (15). Therefore, phosphorylation plays a major role in TAZ regulation. In this study we show that TAZ phosphorylation is also controlled by PP1A and its interacting protein ASPP2. Several lines of evidence support a role of PP1A in TAZ dephosphorylation. First, PP1A is identified in the TAZ complex and the interaction between TAZ and PP1A is confirmed by reciprocal co-IP. Consistently, the PP1 interaction protein ASPP2 is also identified in the TAZ complex. Second, co-expression of PP1A reduces the mobility TAZ shift and decreases TAZ phosphorylation at Ser89 and Ser311 as indicated by Western blotting with the phosphoTAZ (Ser89) and phosphoTAZ(Ser311) antibodies. Third, both phosphatase inhibitor okadaic acid and calyculin A increases TAZ phosphorylation at Ser89 and Ser311. The concentration of okadaic acid needed to increase TAZ phosphorylation is consistent with PP1A being the target phosphatase. Fourth, PP1 efficiently dephosphorylates both Ser89 and Ser311 in TAZ by in vitro reactions. Furthermore, co-expression of PP1A promotes TAZ nuclear localization and TAZ stabilization. Interestingly, YAP dephosphorylation is not similarly controlled by PP1A (Figure S4). It is worth the efforts to identify the phosphatase responsible for YAP dephosphorylation.

Previously, ASPP2 was identified as a PP1 interacting protein, but the biology function of this interaction remained to be explored. Here, we show that ASPP2 promotes TAZ-PP1 interaction by binding with both TAZ and PP1 through different domains, thus enhances dephosphorylation of TAZ. Interestingly, although WW domain mutant significantly abolishes the interaction between TAZ and ASPP2, ASPP2 still enhances dephosphorylation of TAZ, indicating another indirect effect on TAZ. One possibility is ASPP2 can regulate Insulin receptor substrate 1 (35), which has been found to binding with YAP in the cytoplasm(36), to affect YAP/TAZ subcellular location and phosphorylation level.

ASPP2 is a haplo-insufficient tumor
suppressor(37) and it promotes the apoptosis function of p53, similar with p63 and p73(22). Besides p53 family, ASPP2 interacts with numerous proteins, including YAP. However, the biological function of the interaction between ASPP2 and non-p53 family is largely unknown. The interaction between ASPP2 and APP-BP1 inhibits apoptosis in neuronal cells, indicating that ASPP2 could have anti-apoptotic function (30). Here we show that ASPP2 activates TAZ, also indicating an anti-apoptotic function because TAZ inhibits apoptosis. These studies suggest that the biological significance of ASPP2 in cooperating with no-p53 family is cell context dependent.

ASPP2 interacts with TAZ and PP1A via the PY motif and RVXF motif, respectively. ASPP2 belongs to the ASPP family, including pro-apoptotic ASPP1 and anti-apoptotic iASPP (22-24). ASPP1, but not iASPP, also possesses a PY motif and A RVxF motif, implicating ASPP1 may also regulate TAZ phosphorylation level in a manner similar to ASPP2.

TAZ has also been implicated in human tumorigenesis. TAZ is inhibited by the Hippo pathway, which contains well-established human tumor suppressor NF2 (6), and WW45 and Mob that are mutated in human cancer cell lines (38,39). In addition, Overexpression of TAZ in MCF10A cells can promote cell proliferation, EMT, and oncogenesis (6,12,20). We previously demonstrated LATS phosphorylates TAZ to promote its ubiquitin mediated degradation, leading to functional inhibition of TAZ (15). Notably, elevated TAZ expression is observed in more than 20% of breast cancers, especially invasive ductal carcinomas (12). Here we have demonstrated that PP1A coupled with ASPP2 antagonize the function of Lats to regulate the reversible phosphorylation of TAZ. Given the potential role of TAZ in human cancer, it would be interesting to examine the expression of PP1 and ASPP2 in TAZ overexpressed breast cancer tissues. Our studies shed light on the dynamic regulation of TAZ phosphorylation and potential mechanisms of TAZ activation in human cancer.

Acknowledgement
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Figure legends

Figure 1. PP1A dephosphorylates TAZ
A. Mass spectrometry identification of PP1A in Flag-TAZ immunoprecipitation. Cell lysate from 293T cells stabling expressing Flag-TAZ was immunoprecipitated with SBP bead, followed by four times washing and trypsin digestion for further MS analysis. Protein sequence of PP1A peptides identified by mass spectrometry are shown.
B. TAZ binds to PP1A. HA-PP1A/Flag-PP1A was co-transfected with Flag-TAZ/ GFP-TAZ into HEK293T cells as indicated. PP1A and TAZ associations were examined by reciprocal Co-IP as indicated.
C. PP1A dephosphorylates TAZ at Ser89. The indicated plasmids were co-expressed in 293T cells and TAZ phosphorylation at Ser89 was determined by western blot.
D. PP1 but not PP2A dephosphorylates TAZ at Ser89. The indicated plasmids were co-expressed in 293T cells and TAZ phosphorylation at Ser89 was determined by western blot. H248K denotes phosphatase inactive mutant.
E. PP1A dephosphorylates TAZ at Ser89 in vitro. Flag-TAZ was immunoprecipitated from transfected 293T cells, then incubated with indicated amount of recombinant PP1. Phosphorylation of TAZ was detected by pTAZ (Ser89) antibody.
F. Phosphatase inhibitors induce TAZ phosphorylation at Ser89. 293T cells were treated with phosphatase inhibitors as indicated, TAZ phosphorylation at Ser89 was determined by western blot.

Figure 2. PP1A stabilizes TAZ by decreasing the interaction between TAZ and β-TrCP.
A. Phosphatase inhibitors promote proteasome dependent TAZ degradation. 293T cells stably expressing TAZ were treated with OA and MG132 as indicated. The steady state level of TAZ was determined by western blot.
B. PP1A increases TAZ protein levels. Cell lysates from pBABE-Flag-TAZ MCF10A cells expressing vector, PP1A was separated and probed with indicated antibodies.

C. PP1A stabilizes TAZ. pBABE-Flag-TAZ MCF10A cells expressing vector or PP1A were treated CHX (20μg/ml) for indicated times. TAZ protein levels were determined by western blot.

D. PP1A decreases the interaction between TAZ and β-TrCP. The indicated plasmids were co-transfected into 293T cells. Flag-β-TrCP was immunoprecipitated with Flag antibody and the co-precipitated GFP-TAZ was detected by TAZ western blot.

E. Okadaic acid treatment increases the interaction between TAZ and β-TrCP. The indicated plasmids were co-transfected into 293T cells. Flag-TAZ was immunoprecipitated with Flag antibody and the co-precipitated β-TrCP was detected by HA western blot.

F. PP1A decreases TAZ phosphorylation at Ser311. PP1A was co-expressed with TAZ in 293T cells and TAZ phosphorylation at Ser311 was determined by western blot.

G. PP1A dephosphorylates TAZ at Ser311 in vitro. Flag-TAZ was immunoprecipitated from transfected 293T cells followed by incubation with indicated amount of recombinant PP1. Phosphorylation of TAZ was detected by pTAZ (Ser311) antibody.

H. Phosphatase inhibitors induce TAZ phosphorylation at Ser311. 293T cells were treated with phosphatase inhibitors as indicated, TAZ phosphorylation at Ser311 was determined by western blot.

Figure 3. ASPP2 increases the interaction between TAZ and PP1A

A. Both TAZ and PP1A interact with ASPP2. ASPP2 plasmid was co-transfected into 293T cells as indicated. Flag-TAZ or Flag-PP1A was immunoprecipitated with Flag antibody and the co-precipitated ASPP2-His was detected by His western blot.

B. ASPP2 facilitates the interaction between TAZ and PP1A. The indicated plasmids were co-transfected into 293T cells. Flag-PP1A was immunoprecipitated with Flag antibody and the co-precipitated HA-TAZ or ASPP2-His was detected by HA or His western blot.

C. TAZ requires its WW domain to interact with ASPP2. The indicated plasmids were co-transfected into 293T cells. Flag-TAZ was immunoprecipitated with Flag antibody and the co-precipitated ASPP2-His was detected by His western blot.

D. The WW domain in TAZ is required for ASPP2 to enhance the interaction between TAZ and PP1A. The indicated plasmids were co-transfected into 293T cells. Flag-TAZ was immunoprecipitated with Flag antibody and the co-precipitated ASPP2-His or HA-PP1A was detected by His or HA western blot.

E. The WW domain in TAZ is important for its dephosphorylation by ASPP2. The indicated plasmids were co-expressed in 293T cells and TAZ phosphorylation at Ser89 was determined by western blot.

F. The PP1 binding motif RVXF in ASPP2 is required for it to promote dephosphorylation of TAZ Ser89. The indicated plasmids were co-expressed in 293T cells and TAZ phosphorylation at Ser89 was determined by western blot.

G. ASPP2 knockdown increases TAZ phosphorylation at Ser89. Three ASPP2 RNAi oligos were individually or combined transfected into A375 cells as indicated. ASPP2 and TAZ phosphorylation at Ser89 levels were determined by Western blot.
**Figure 4. PP1A and ASPP2 promote TAZ nuclear localization**

A. PP1A decreases the interaction between TAZ and 14-3-3. The plasmids were co-transfected into 293T cells as indicated. Flag-TAZ was immunoprecipitated with Flag antibody and the co-precipitated Myc-14-3-3 was detected by Myc western blot.

B. Phosphatase inhibitors increase the interaction between TAZ and 14-3-3. The plasmids were co-transfected into 293T cells as indicated. Flag-TAZ was immunoprecipitated with Flag antibody and the co-precipitated HA-14-3-3 was detected by HA western blot.

C. The PP1 binding motif RVXF is required for ASPP2 to inhibit the interaction between TAZ and 14-3-3. The plasmids were co-transfected into 293T cells as indicated. Flag-TAZ was immunoprecipitated with Flag antibody and the co-precipitated HA-14-3-3 was detected by HA western blot.

D. PP1A and ASPP2 promote TAZ nuclear localization. The plasmids were co-transfected into 293T cells as indicated. Cytoplasmic and nuclear fractions were separated for western blot analysis as indicated.

E. Phosphatase inhibitor treatment increases TAZ cytoplasmic sequester. BT549 cells were treated with 100nM OA for 2h. Immunofluorescence was performed to determine TAZ cytoplasmic/nuclear distribution with TAZ antibody from BD.

**Figure 5. PP1A and ASPP2 promote TAZ target gene expression**

A. PP1A increases N-Cadherin, CTGF and decreases E-Cadherin expression in pBABE-Flag-TAZ MCF10A cells. Total RNA was extracted from MCF10A-vector, MCF10A-pBABE-Flag-TAZ/pQCXIH-vec, and MCF10A-pBABE-Flag-TAZ/pQCXIH-PP1A cells and Quantitative-PCR was performed to determine E-Cadherin & N-Cadherin expression, and CTGF expression. All data are normalized to GAPDH.

B. ASPP2 knock down decreases CTGF expression. ASPP2 was depleted in TAZ WT and 4SA MCF10A cells using siRNA against human ASPP2. The efficiency of ASPP2 knock down and down-regulation of CTGF was determined by relative quantitative PCR.
A. PP1A Peptides identified by MS/MS

6KLNLDLSIIGRL17
55REIFLSQPILELEAPLKI73
258RAHQVVEDGEFFAKR273
317KYGQFSGLNPGGRP330

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E.

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F.

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Liu, et al, Figure 1
Liu, et al, Figure 2
Liu, et al, Figure 3
Liu, et al, Figure 4
Liu, et al, Figure 5
PP1 Cooperates with ASPP2 to dephosphorylate and activate TAZ
Chen-Ying Liu, Xianbo Lv, Tingting Li, Yanping Xu, Xin Zhou, Shimin Zhao, Yue Xiong, Qun-Ying Lei and Kun-Liang Guan

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