Structural and functional analysis of the YAP-binding domain of human TEAD2

Wei Tian, a Jiantzhong Yu, a Diana R. Tomchick, a Duojia Pan, b,c and Xuelian Luo b,c

aDepartments of Pharmacology and Biochemistry, The University of Texas Southwestern Medical Center, Dallas, TX; bDepartment of Molecular Biology and Genetics, Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD; and cDepartment of Molecular Biology, The University of Texas Southwestern Medical Center, Dallas, TX.

Edited by Johann Deisenhofer, The University of Texas Southwestern Medical Center, Dallas, TX, and approved March 1, 2010 (received for review January 8, 2010)

The Hippo pathway controls organ size and suppresses tumorigenesis in metazoans by blocking cell proliferation and promoting apoptosis. The TEAD1-4 proteins (which contain a DNA-binding domain but lack an activation domain) interact with YAP (which lacks a DNA-binding domain but contains an activation domain) to form functional heterodimeric transcription factors that activates proliferative and prosurvival gene expression programs. The Hippo pathway inhibits the YAP-TEAD hybrid transcription factors by phosphorylating and promoting cytoplasmic retention of YAP. Here we report the crystal structure of the YAP-binding domain (YBD) of human TEAD2. TEAD2 YBD adopts an immunoglobulin-like β-sandwich fold with two extra helix-turn-helix inserts. NMR studies reveal that the TEAD-binding domain of YAP is natively unfolded and that TEAD binding causes localized conformational changes in YAP. In vitro binding and in vivo functional assays define an extensive conserved surface of TEAD2 YBD as the YAP-binding site. Therefore, our studies suggest that a short segment of YAP adopts an extended conformation and forms extensive contacts with a rigid surface of TEAD. Targeting a surface-exposed pocket of TEAD might be an effective strategy to disrupt the YAP-TEAD interaction and to reduce the oncogenic potential of YAP.

Expression of mammalian MST2, LATS1, and MOBI1 genes in flies functionally rescues the phenotypes of the corresponding Hpo, Wts, and Mst1 mutants (8–12, 16, 21). Similar to the fly pathway, the effectors of the mammalian Hippo pathway are transcription factors, including YAP (Yes-associated protein), and TEAD, the mammalian homologs of Yki and Sd, respectively (16, 18, 22, 23). There are four closely related human TEAD proteins, TEAD1-4. All TEAD proteins contain an N-terminal DNA-binding TEA (TFE-1, TEC1, and AbaA) domain and a C-terminal YAP-binding domain (YBD) (Fig. 1A). YAP has an N-terminal TEAD-binding domain and a C-terminal transcriptional activation domain. Through direct physical interaction, TEAD YBD recruits YAP to promoters of target genes, where it turns on gene expression. When the Hippo pathway is activated, LATS1/2 phosphorylates YAP on S127 and promotes its association with 14-3-3 and cytosolic retention, thus inhibiting the activities of the hybrid transcription factors formed between YAP and TEAD1-4.

The Hippo pathway has been implicated in human cancers. In particular, several lines of evidence indicate YAP as an oncogene. The YAP gene is amplified in several human cancers. The YAP protein is frequently overexpressed in human cancers (12, 24–26). Overexpression of YAP in nontransformed human MCF10A cells induces the epithelial-mesenchymal transition, a hallmark of tumorigenic transformation (24). Overexpression of YAP in mouse liver causes dramatic liver overgrowth and eventually tumor formation (11, 27). The TEAD proteins are major partners of YAP and collaborate with it to regulate the expression of genes that promote cell proliferation and inhibit apoptosis. Thus, understanding how TEAD interacts with YAP will provide insights into how the Hippo pathway regulates the YAP-TEAD transcription factors and may ultimately lead to strategies that can disrupt the tumor-promoting YAP-TEAD interactions.

Toward this goal, we have determined the crystal structure of the YAP-binding domain of human TEAD2. We have further analyzed the interactions between TEAD and YAP using in vitro binding assays, in vivo luciferase assays, and NMR spectroscopy. Our studies pinpoint a surface-exposed pocket of TEAD YBD that is critical for YAP binding. Targeting this pocket chemically might be an effective strategy to disrupt the YAP-TEAD interactions and to attenuate the function of YAP.

Results and Discussion

Structure Determination of the YAP-Binding Domain of TEAD. The structure of the TEA DNA-binding domain of TEAD proteins has been determined previously (28). The structure of the

*Author contributions: W.T., D.P., and X.L. designed research; W.T., J.Y., and X.L. performed research; D.R.T., D.P., and X.L. analyzed data; and X.L. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 3L15).

1To whom correspondence may be addressed. E-mail: xuelian.luo@utsouthwestern.edu or djpan@jhu.edu.
YBD of TEAD is unknown, however. We have determined the structure of human TEAD2 YBD containing residues 217–447 by the single anomalous dispersion (SAD) method from crystals of the seleno-methionine-labeled TEAD217–447 protein (Table 1). TEAD2 YBD adopts an immunoglobulin (IgG)-like fold with βββ antiparallel strands, including β1, β2, β5, β8, and β9, while the other contains seven parallel and antiparallel strands, including β3, β4, β6, β7, and β10–12. In addition to the two main β-sheets, TEAD2 YBD contains two helix-turn-helix motifs that are absent in the IgG-like fold. One helix-turn-helix motif consists of aα and αβ and connects β3 and β4. This motif along with the β2–β3 loop encircles the C-terminal β12 strand, forming an unusual pseudoknot structure. The second helix-turn-helix motif consists of αC and αD and connects β9 and β10. This motif caps the opening at one end of the β-sandwich.

Comparison between TEAD2 YBD and known structures in the Protein Data Bank (PDB) using the Dali server reveals that TEAD2 YBD is structurally most closely related to phosphodiesterase δ (PDEδ) (PDB ID code 1KSHB) with a Z score of 10.1 and a rmsd of 2.5 Å. Superposition of the TEAD2 YBD and PDEδ structures reveals that the main β-sheets of the two molecules overlay well (Fig. 1C). Their major difference is that PDEδ has an N-terminal α-helix, whereas TEAD2 YBD has two helix-turn-helix inserts. Interestingly, PDEδ binds to the small GTPase Arf-like 2 (Arf2) through an edge-on β-β interaction, with one of its edge strands paring with the edge strand in Arf2 (Fig. 1C) (29). Based on the structural similarity, TEAD2 YBD might also use its edge strands to interact with YAP or other yet unidentified binding partners.

The TEAD-Binding Domain (TBD) of YAP Is Natively Unfolded. We next studied the interaction between TEAD2 YBD and YAP using NMR. Previous studies have mapped the TBD of YAP to a small N-terminal region containing residues 48–102 (Fig. L4) (30, 31). We expressed a large fragment of YAP (residues 2–268) containing the TBD and the two WW domains, labeled it with 15N, and acquired a 2D 1H/15N heteronuclear single quantum correlation (HSQC) spectrum (Fig. 2A). The HSQC spectrum of YAP2–268 contains two sets of peaks. One set of peaks is well dispersed and has intensities comparable to the peaks of several tryptophan side chains. These peaks presumably belong to the two WW domains of YAP. The other set of peaks is strong in intensity and clusters around 7.8–8.5 ppm in the 1H dimension. These peaks belong to residues with flexible random coil conformations. Therefore, a segment of YAP2–268 is natively unfolded. We next prepared 15N-labeled YAP2–268 in complex with TEAD2 YBD and acquired an HSQC spectrum on it. An overlay of the HSQC spectra of free YAP2–268 and the YAP2–268–TEAD2 YBD complex reveals that the signals belonging to the WW domains of YAP remain largely unaffected by TEAD2 YBD binding (Fig. 2B). This indicates that the WW domains of YAP are not involved in TEAD binding. By contrast, about 30 sharp and strong signals of YAP2–268 either disappear or become much weaker.

### Table 1. Data collection, structure determination, and refinement

<table>
<thead>
<tr>
<th>Data collection</th>
<th>SeMet (peak)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>C2</td>
</tr>
<tr>
<td>Energy (eV)</td>
<td>12,655.6</td>
</tr>
<tr>
<td>Resolution range (Å)</td>
<td>50.0–2.00</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>34,385 (1,250)</td>
</tr>
<tr>
<td>Multiplicity</td>
<td>4.9 (3.8)</td>
</tr>
<tr>
<td>Data completeness (%)</td>
<td>96.1 (70.9)</td>
</tr>
<tr>
<td>Rmerge (%)</td>
<td>10.1 (71.1)</td>
</tr>
<tr>
<td>animal B value, Å2</td>
<td>10.5 (1.8)</td>
</tr>
<tr>
<td>Wilson B value, Å2</td>
<td>31.9</td>
</tr>
<tr>
<td>Phase determination</td>
<td>Selenium (8 of 8 possible sites)</td>
</tr>
<tr>
<td>Figure of merit, 50–2.00 Å</td>
<td>0.36</td>
</tr>
</tbody>
</table>

### Data for the outermost shell are given in parentheses.

*Bijvoet pairs were kept separate for data processing.

\[R_{merge} = \frac{\sum \sum |I_{h;i} - \langle I_h \rangle|}{\sum \sum I_{h;i}} \]

where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.

†As defined by the validation suite MolProbity.

### Notes

- The data were collected at 12.655.6 eV, with a resolution range of 50.0–2.0 Å.
- The data completeness was 96.1% (70.9%) for the 15N-labeled YAP2–268.
- The Rmerge was 10.1% (71.1%) for the 15N-labeled YAP2–268.
- The Wilson B value was 10.5 Å2 (1.8 Å2).

### References

- Tian et al., www.pnas.org/cgi/doi/10.1073/pnas.1000293107
- Previous studies have mapped the TBD of YAP to a small N-terminal region containing residues 48–102 (Fig. L4) (30, 31).

---

**Fig. 1.** Structure of human TEAD217–447. (A) Schematic drawing of the domain organization for human YAP and TEAD2 proteins. The residue numbers for different domain boundaries are labeled. TBD: TEAD-binding domain; AD: transcriptional activation domain; TEA: DNA-binding TEA domain; YBD: YAP-binding domain. (B) Ribbon diagram of TEAD217–447 in two views. The main organization for human YAP and TEAD2 proteins. The residue numbers for different domain boundaries are labeled. (C) Left) Ribbon diagram of the PDEδ-Arl2 complex. The PDEδ molecule is colored in wheat and the Arl2 molecule is colored in green. (Right) Superposition of PDEδ and TEAD217–447. All structural figures were generated with PyMOL.
Weaker upon TEAD binding. These residues likely adopt ordered conformation when bound to TEAD2 YBD. Therefore, TEAD binding induces localized conformational changes of YAP. A small segment of YAP is directly involved in TEAD binding.

We thus synthesized two peptides within the TBD of YAP: One peptide contains residues 61–100 of YAP while the other contains residues 67–97 and measured their binding affinity to TEAD2 YBD using isothermal titration calorimetry (ITC) (Fig. 2B). Consistent with our NMR data, the YAP61–100 peptide bound to TEAD2 YBD with a dissociation constant (Kd) of 96 nM and a stoichiometry of 0.94. Therefore, a short 40mer YAP peptide is sufficient to bind TEAD2 YBD with relatively high affinity to form a 1∶1 complex. On the other hand, we could not detect appreciable binding between TEAD2 YBD and the smaller YAP67–97 peptide with ITC. Furthermore, the ITC experiment also revealed that YAP2–268 used in the NMR studies binds to TEAD2 YBD with a dissociation constant (Kd) of 96 nM and a stoichiometry (N) of 0.97 similar to those of the YAP61–100 peptide (Fig. 2C). Thus, residues 61–100 represent the essential TEAD-binding domain of YAP.

Mapping the YAP-Binding Site of TEAD2 YBD. Because we failed to obtain diffracting crystals of TEAD2 YBD bound to various YAP fragments containing the minimal TBD as defined above, we used structure-based mutagenesis to map the YAP-binding site on TEAD2 YBD. We first aligned the sequences of TEAD YBD from various species ranging from Caenorhabditis elegans to human and displayed the side chains of surface-exposed residues that are identical in TEAD YBD from all species on the ribbon diagram (Fig. 3). These conserved residues form a contiguous surface on one face of TEAD2 YBD that contains β7, αC, αD, and the back of stands β4, β11, and β12. The other face of TEAD2 YBD contains few conserved residues. A conserved tyrosine (Y442 in TEAD2 YBD) in human TEAD1 is mutated to histidine in patients with a rare eye disorder called Sveinsson’s chorioretinal atrophy (32). This mutation has been shown previously to disrupt the YAP-TEAD interaction (33). Intriguingly, Y442 is surface exposed and is located at the back of strand β12 (Fig. 3B and C). Therefore, this conserved surface of TEAD2 YBD might be involved in YAP binding.

We next systematically mutated these surface-exposed conserved residues of TEAD2 YBD in the context of full-length TEAD2 and tested the binding between these mutants and YAP2–268. To do so, we expressed and purified GST-YAP2–268, immobilized this fusion protein on glutathione-agarose beads, and incubated these beads with in vitro translated 35S-labeled TEAD2 mutants. After washing, the proteins bound to beads were resolved on SDS-PAGE and visualized using a phosphoimager (Fig. 4A and B). As expected, wild-type (WT) TEAD2 bound specifically to GST-YAP2–268, but not to the GST control. Consistent with previous reports, the TEAD2 Y442H mutation abolished YAP binding. Mutations of the majority of these conserved residues resulted in a reduction of YAP binding by 10–1000 fold, similar to a recent study (34).

Correlation of YAP Binding to the TEAD Domain Homology and in Vitro Mutations. We next compared the YAP binding to TEADs with various degrees of sequence conservation. As shown in Fig. 5A, the YAP binding to WT TEAD2 YBD is sufficient to induce the formation of YAP-TEAD complexes. In contrast, all the mutants except Y442N bind to GST-YAP2–268 with a significantly reduced Kd, indicating that residue Y442 is critical for YAP binding. These results are consistent with those of the NMR data. As expected, the wild-type (WT) TEAD2 YBD bound to GST-YAP2–268, but not to the GST control. Consistent with previous reports, the TEAD2 Y442H mutation abolished YAP binding. Mutations of the majority of these conserved residues resulted in a reduction of YAP binding by 10–1000 fold, similar to a recent study (34).
residues weakened the binding of YAP to TEAD2, confirming the involvement of this conserved surface in YAP binding.

A total of 10 mutant constructs (including Y442H) reduced YAP binding to below 10% of that of the wild-type TEAD2. Nine out of these 10 mutant constructs affected residues that formed a surface-exposed pocket, including E267, I274, K277, L299/K301, W303, E404/N405, V427/E429, Y442, and L444 (Fig. 4C).

Strands β4, β11, and β12 form the base of this pocket, whereas β3, αA, and αD form the walls. The residues in the center of the pocket are hydrophobic, whereas many residues lining the periphery of the pocket are polar or charged, including E267, K277, K301, E404, N405, and E429 (Fig. 4D). Therefore, despite having an extensive YAP-binding surface, this pocket on TEAD2 YBD represents a major anchoring point for YAP and contributes a large fraction of their binding energy. Strand β7 and the surface formed by αC and αD contribute the rest of the binding surface and further strengthen YAP binding.

As discussed above, YAP is an oncogene and is frequently overexpressed in human cancers. Inhibiting YAP might be an effective way to block the proliferation and to induce apoptosis in cancer cells. TEAD proteins are the major partners of YAP in transcripational activation. Disrupting the YAP-TEAD interaction is expected to significantly weaken the transforming function of YAP. YAP binding to TEAD requires a surface pocket on TEAD that is formed at the back of the seven-stranded β-sheet and lined with αA and αD. This pocket contains both hydrophobic and charged residues, making it feasible to design or to screen for chemical compounds that selectively bind to this site. This pocket is highly conserved in TEAD1-4. These compounds might be able to disrupt the YAP-TEAD1-4 interactions and have therapeutic potential.

The YAP-TEAD Interaction Is Required for Transcriptional Activation.

To further confirm the validity of the YAP-binding site on TEAD2 in cells, we constructed plasmids encoding the wild-type TEAD2. Nine out of these 10 mutant constructs affected residues that formed a surface-exposed pocket, including E267, I274, K277, L299/K301, W303, E404/N405, V427/E429, Y442, and L444 (Fig. 4C). Strands β4, β11, and β12 form the base of this pocket, whereas β3, αA, and αD form the walls. The residues in the center of the pocket are hydrophobic, whereas many residues lining the periphery of the pocket are polar or charged, including E267, K277, K301, E404, N405, and E429 (Fig. 4D). Therefore, despite having an extensive YAP-binding surface, this pocket on TEAD2 YBD represents a major anchoring point for YAP and contributes a large fraction of their binding energy. Strand β7 and the surface formed by αC and αD contribute the rest of the binding surface and further strengthen YAP binding.

As discussed above, YAP is an oncogene and is frequently overexpressed in human cancers. Inhibiting YAP might be an effective way to block the proliferation and to induce apoptosis in cancer cells. TEAD proteins are the major partners of YAP in transcriptional activation. Disrupting the YAP-TEAD interaction is expected to significantly weaken the transforming function of YAP. YAP binding to TEAD requires a surface pocket on TEAD that is formed at the back of the seven-stranded β-sheet and lined with αA and αD. This pocket contains both hydrophobic and charged residues, making it feasible to design or to screen for chemical compounds that selectively bind to this site. This pocket is highly conserved in TEAD1-4. These compounds might be able to disrupt the YAP-TEAD1-4 interactions and have therapeutic potential.

The YAP-TEAD Interaction Is Required for Transcriptional Activation.

To further confirm the validity of the YAP-binding site on TEAD2 in cells, we constructed plasmids encoding the wild-type or mutant TEAD2 proteins fused to the Gal4 DNA-binding domain at their N termini and transfected 293 cells with these plasmids together with a Gal4-driven luciferase reporter plasmid in the presence or absence of YAP (Fig. 5). Gal4 DNA-binding domain or Gal4-TEAD2 WT alone did not activate the expression
of the luciferase reporter. Cotransfection of YAP and Gal4-TEAD2 WT greatly increased the luciferase activity. By contrast, cotransfection of YAP and the Gal4-TEAD2 L299A/K301E, V427A/E429K, or Y442H mutants that lost their ability to bind YAP failed to activate the expression of the luciferase reporter. As a control, the Gal4-TEAD2 S349A/F350A mutant that retained YAP binding activated luciferase expression as did Gal4-TEAD2 WT. Therefore, these results indicate that the YAP-TEAD interaction is required for its ability to activate transcription. The YAP-binding site on TEAD2 YBD mapped by in vitro binding assays is also critical for YAP function in living cells.

Conclusions

The Hippo pathway controls tissue homeostasis in multicellular organisms by restricting cell proliferation and promoting cell death. Malfunction of the Hippo pathway results in hyperplasia and cancer. One important output of this pathway is the inhibition of the YAP transcription coactivator, which involves the phosphorylation and cytoplasmic retention of YAP and the disruption of the binding of YAP to its major cellular partners, the TEAD proteins. In this paper, we have determined the structure of the YAP-binding domain of human TEAD2 and studied the YAP-TEAD interactions with a combination of biophysical, biochemical, and cellular assays. Our results suggest that a short natively unfolded segment of YAP binds to an extensive surface on TEAD and adopts an ordered conformation. Despite having an extensive interface, the YAP-TEAD interaction requires a surface pocket that has both hydrophobic and hydrophilic characters. Chemical compounds that selectively bind to this pocket may disrupt the YAP-TEAD interactions and may have therapeutic potential.

Materials and Methods

Plasmids, Protein Expression, and Purification. The coding region of human TEAD2 was amplified by PCR and cloned into a modified pET28 vector (EMD Biosciences) that also included a tobacco etch virus (TEV) protease cleavage site. The TEAD2 mutants were generated with the QuikChange mutagenesis kit (Stratagene). All constructs were verified by DNA sequencing. The pET28-TEAD2 plasmid was transformed into the Escherichia coli strain BL21(DE3) to produce N-terminal His₆-tagged TEAD2 protein. TEAD2 was purified with Ni²⁺-NTA agarose resin (Qiagen) and cleaved with TEV protease to remove the His₆ tag. The protein was further purified by anion exchange chromatography followed by size exclusion chromatography (GE Healthcare). Purified TEAD2 was concentrated to 12 mg/ml in a buffer containing 20 mM Tris (pH 8.0), 100 mM NaCl, 2 mM MgCl₂, 1 mM Tris(2-carboxyethyl)phosphine (TCEP), and 5% glycerol. The seleno-methionine labeled TEAD2 was produced using the methionine biosynthesis inhibition method (34).

To generate the pcDNAGal4DBD construct, the cDNA encoding the Gal4 DNA-binding domain (DBD) (1-147) was cloned into the pcDNA 3.1 vector. The wild-type or mutant TEAD2 sequences were inserted into the pcDNA-Gal4DBD vector in frame with Gal4DBD to generate Gal4-TEAD2 WT or Gal4-TEAD2 mutant constructs.

Crystallization, Data Collection and Structure Determination. TEAD2 was crystallized at 20 °C using the hanging-drop vapor-diffusion method with a reservoir solution containing 0.1 M Hepes (pH 7.4) and 2.8 M sodium formate. The crystals were cryoprotected with reservoir solution supplemented with 25% glycerol and then flash-cooled in liquid nitrogen. Crystals diffracted to a minimum Bragg spacing (d_min) of about 2.0 Å and exhibited the symmetry of space group C2 with cell dimensions of a = 121.1 Å, b = 61.6 Å, c = 80.5 Å, and β = 117.3° and contained two molecules per asymmetric unit. Diffraction data were collected at beamline 19-BM (SBC-CAT) at the Advanced Photon Source (Argonne National Laboratory) and processed with HKL3000 (35). Phases were obtained from a selenium-SAD experiment using X-rays at an energy near the selenium K absorption edge. Phenix AutoSol was used to identify the selenium sites and calculate density modified experimental maps (36). A total of eight refined sites were found, and the experimental
density map showed clear features of the protein backbone and well defined side chains. Automated building with Phenix AutoBuild resulted in a model containing 366 sequence assigned residues when refined against the experimental phases. The remaining 31 residues were manually built in COOT and refined in Phenix (37). The final model (R_{work} = 18.8\%, \ R_{free} = 24.1\%) contains 397 residues, 160 water molecules, and 3 glycerol molecules. MolProbity was used for structure validation and indicates all residues are in the Ramachandran favored/allowed regions (38). Data collection and structure refinement statistics are summarized in Table 1.

Isotermal Titration Calorimetry (ITC). ITC was performed with a VP-ITC titration calorimeter (MicroCal Inc.) at 20 °C. Calorimetric measurements were carried out with purified TEAD2\(^{21–47}\) and YAP2\(^{22–28}\) plus two synthetic peptides corresponding to residues 61–100 (SETDLEALFNAVMPKTANVPQ-TVMPRLKLFSDPFPKE) and 67–97 (ALNAVMPKTANVPQ-TVMPRLKLFSDPFPKE) of human YAP. For each titration experiment, 2 mL of 6 \mu M TEAD protein in a buffer containing 20 mM Tris (pH 8.0), 100 mM KCl, 2 mM MgCl\(_2\), and 0.5 mM TCEP were added to the calorimeter cell. The YAP peptides (0.05–0.1 \mu M) in the same buffer were injected with 35 portions of 8 \mu L with an injection syringe. Binding parameters were evaluated using the Origin software package provided with the instrument.

NMR Spectroscopy. All NMR spectra were acquired at 30 °C on a Varian Unity Inova 800-MHz spectrometer using H\(_2\)O-D\(_2\)/O 95: 5 (v/vol) as the solvent. Samples typically contained 0.1 mM \(^{15}\)N-labeled protein in the NMR buffer consisting of 20 mM sodium phosphate (pH 6.8), 100 mM KCl, 2 mM MgCl\(_2\), and 1 mM DTT.

In Vitro Protein Binding Assays. To assay the binding between human TEAD2 and YAP proteins, the full-length WT or mutant TEAD2 was translated in reticulocyte lysate in the presence of \(^{35}\)S-methionine. Purified GST-YAP\(^{22–28}\) was reacting with glutathione-agarose beads (GE Healthcare), incubated with \(^{35}\)S-labeled TEAD proteins, and washed three times with Tris buffered saline containing 0.05% Tween. The proteins retained on the beads were analyzed by SDS-PAGE followed by autoradiography. Glutathione-agarose beads bound to GST were used as controls.

Luciferase Assay. For the luciferase assay, the Gal4-TEAD2 WT or Gal4-TEAD2 mutant plasmids were transfected in triplicates in 293 cells along with a luciferase reporter plasmid with or without YAP. The luciferase assay was performed using the Dual Luciferase Assay System (Promega) and a FLUOstar Luminometer (BMG Lab Technologies).

ACKNOWLEDGMENTS. We thank Xuewu Zhang for help in data collection and Hongtao Yu for discussion and critical reading of the manuscript. Results shown in this paper are derived from work performed at Argonne National Laboratory, Structural Biology Center at the Advanced Photon Source. Argonne is operated by University of Chicago Argonne, LLC, for the U.S. Department of Energy, Office of Biological and Environmental Research under Contract DE-AC02-06CH11357. This work was supported in part by National Institutes of Health Grant GM085004 (to X.L.) and Grant EY015708 (to D.P.).