Supplemental Information

Autopalmitoylation of TEAD Proteins Regulates Transcriptional Output of Hippo Pathway

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Supplemental Results

TABLES

Supplementary Table 1. Data collection and refinement statistics (molecular replacement).

	$TEAD2^{217-447}$–PLM
Data collection	
Space group	C2
Cell dimensions	
a, *b*, *c* (Å)	122.49, 61.15, 80.31
a, b, g (°)	90.00, 117.17, 90.00
Resolution (Å)*	40.00 – 2.04 (2.08 – 2.04)
R_{merge} (%)*	5.5 (50.8)
I / sI*	26.3 (2.36)
Completeness (%)*	95.9 (87.0)
Redundancy*	4.0 (3.6)
Refinement	
Resolution (Å)*	36.1 – 2.05 (2.12 – 2.05)
No. reflections	28795
R_{work} / R_{free}	18.2 / 22.8
No. atoms	
Protein	3308
Ligand/ion	36
Water	155
B-factors (Å^2)	
Protein	38.4
Ligand/ion	27.1
Water	41.6
R.m.s. deviations	
Bond lengths (Å)	0.014
Bond angles (°)	1.41

*Highest-resolution shell is shown in parentheses.

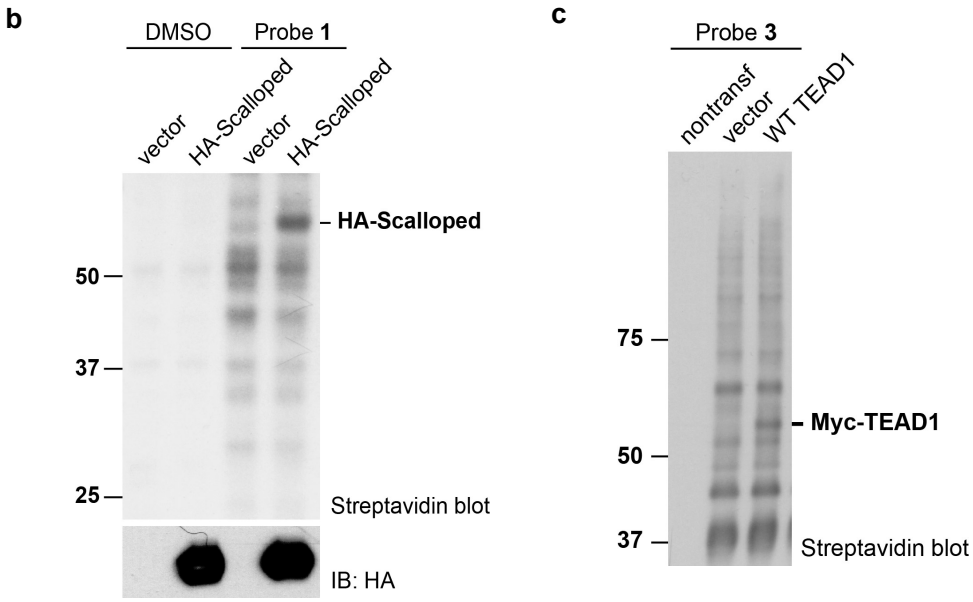
FIGURES

a

Probe **2** Labeled Proteins

TEAD1	
# Peptide spectra	**Unique Peptide Sequence**
2	K.GPQNAFFLVK.F
12	R.KIILSDEGK.M
9	K.VETEYAR.F
4	R.LVEFSAFLEQQR.D

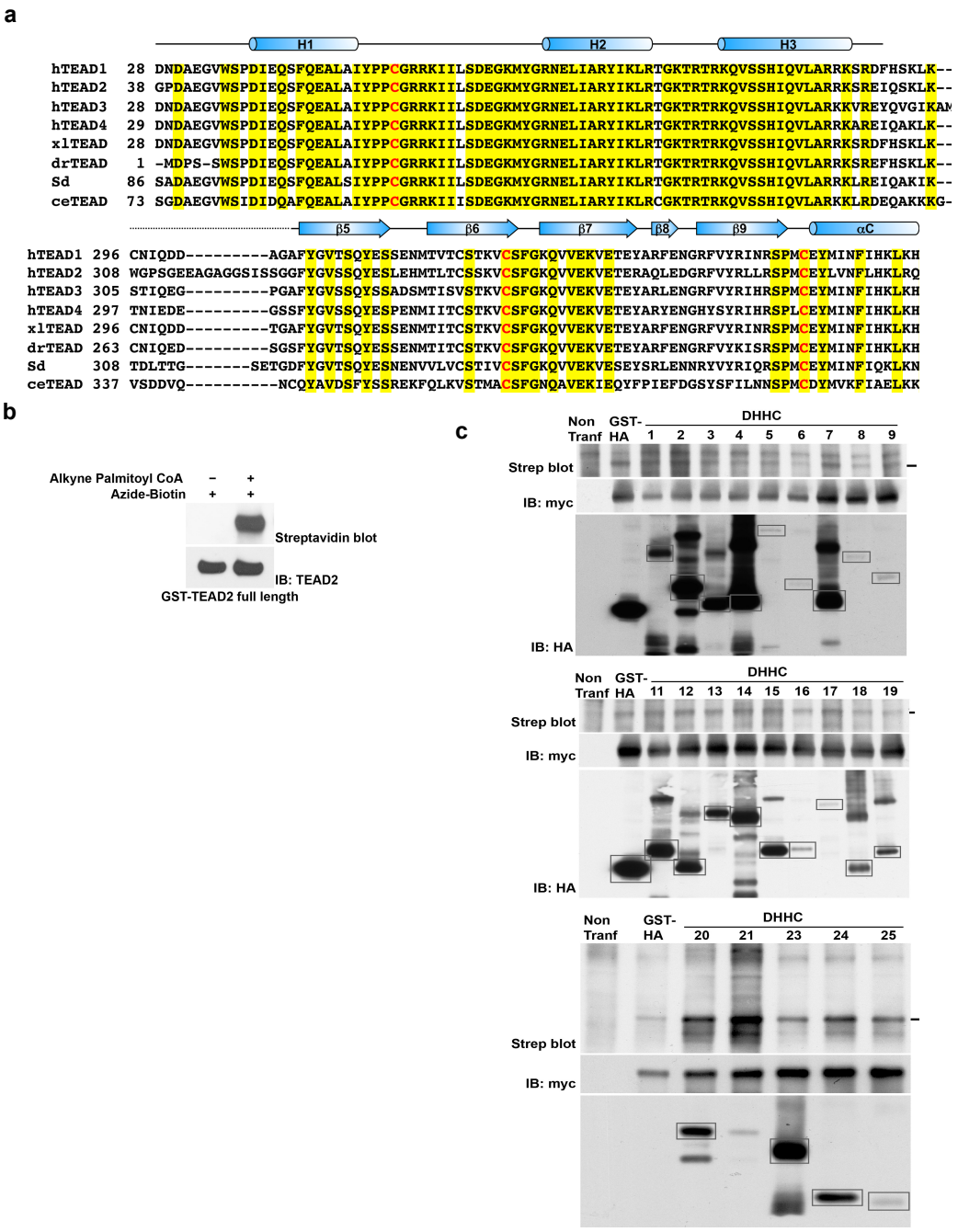
TEAD3	
# Peptide spectra	**Unique Peptide Sequence**
2	K.FSPPSPLPQAVFSTSSR.F
2	K.GPPNAFFLVK.F



Supplementary Figure 1. TEAD transcription factors are palmitoylated.
(**a**) Unique peptide sequences of TEAD1 and 3 identified from proteomic profiling using Probe **2**. The mass spec results were adapted from our previous studies [1].

(**b**) Scalloped is palmitoylated. HEK293A cells were transfected with empty vector or wild type HA-Scalloped. Cells were labeled with 50 μM Probe 1 overnight, lysed and followed by click chemistry reaction. Proteins were resolved by SDS-PAGE and biotin-linked proteins were detected by streptavidin blot. See **Supplementary Figure 13** for the full images of the blots.

(**c**) Clickable analogue of cerulenin (**3**) labels TEAD1. HEK293A cells were transfected with nothing, empty vector or wild type Myc-TEAD1. Cells were labeled with 5 μM of **3** overnight, lysed and followed by click chemistry reaction. Proteins were resolved by SDS-PAGE and biotin-linked proteins were detected by streptavidin blot.

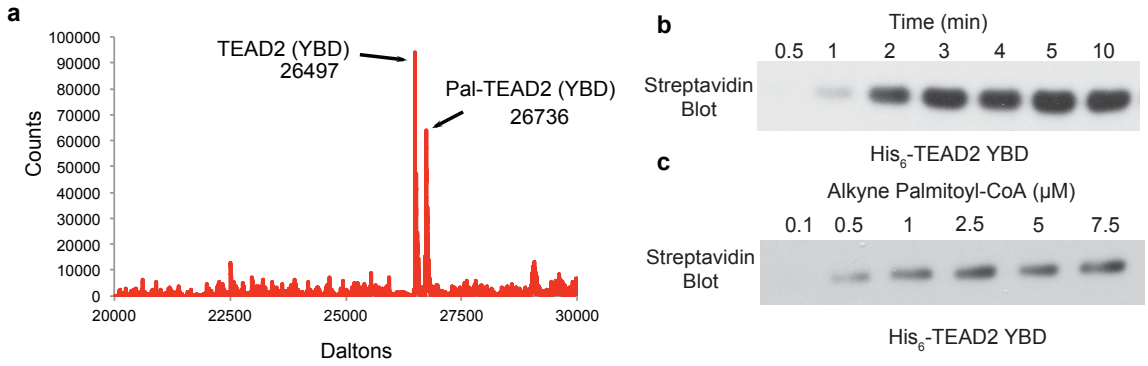


Supplementary Figure 2. TEAD is autopalmitoylated at evolutionarily conserved cysteine residues.

(**a**) Sequence alignment of TEAD family transcription factors across species. The sequences of human TEAD1 – 4, *Xenopus laevis* TEAD, *Danio rerio* TEAD, *Drosophila* Scalloped and *C. elegans* TEAD were aligned. Evolutionarily conserved cysteine residues are highlighted in red. Secondary structure of TEAD2 is shown on top of the sequences.

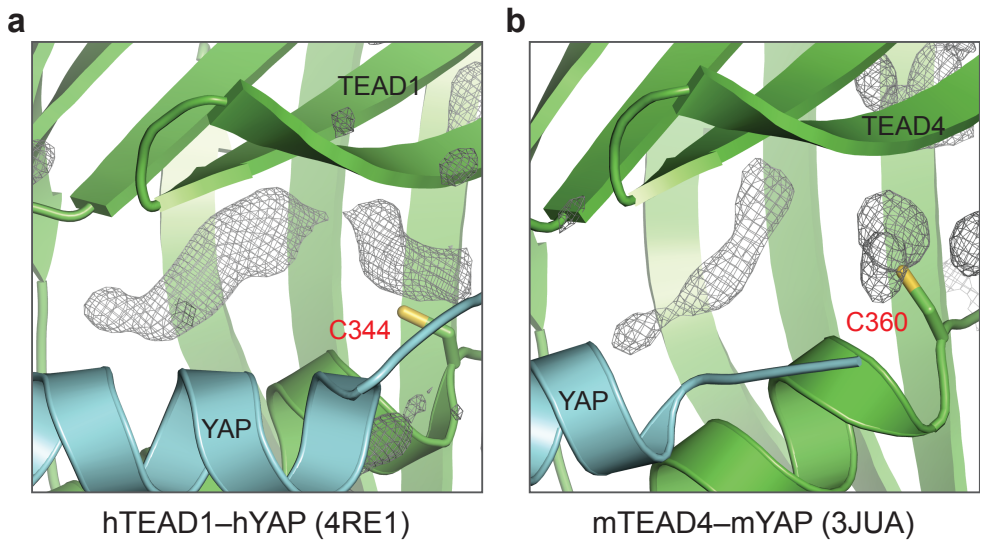
(**b**) *In vitro* palmitoylation of full length GST-TEAD2. Recombinant full-length human GST-TEAD2 (abcam) protein was incubated with 50 μM alkyne palmitoyl CoA for 2 h at 25ºC. Protein was processed further for Click chemistry reaction. Biotinylation of TEAD2 was detected by streptavidin blot and total protein was detected using a TEAD2-specific antibody. See **Supplementary Figure 14** for the full images of the blots.

(**c**) Overexpression of DHHC-PATs did not enhance TEAD1 palmitoylation. HEK293A cells were transfected with nothing, control vector, wild type Myc-TEAD1 and/or HA-DHHC. Cells were labeled with 50 μM Probe **1** overnight, lysed and followed by click chemistry reaction. Proteins were resolved by SDS-PAGE and TEAD protein was detected by streptavidin blot and anti-c-myc antibody. Total DHHC protein was detected by anti-HA antibody. See **Supplementary Figure 15** for the full images of the blots.

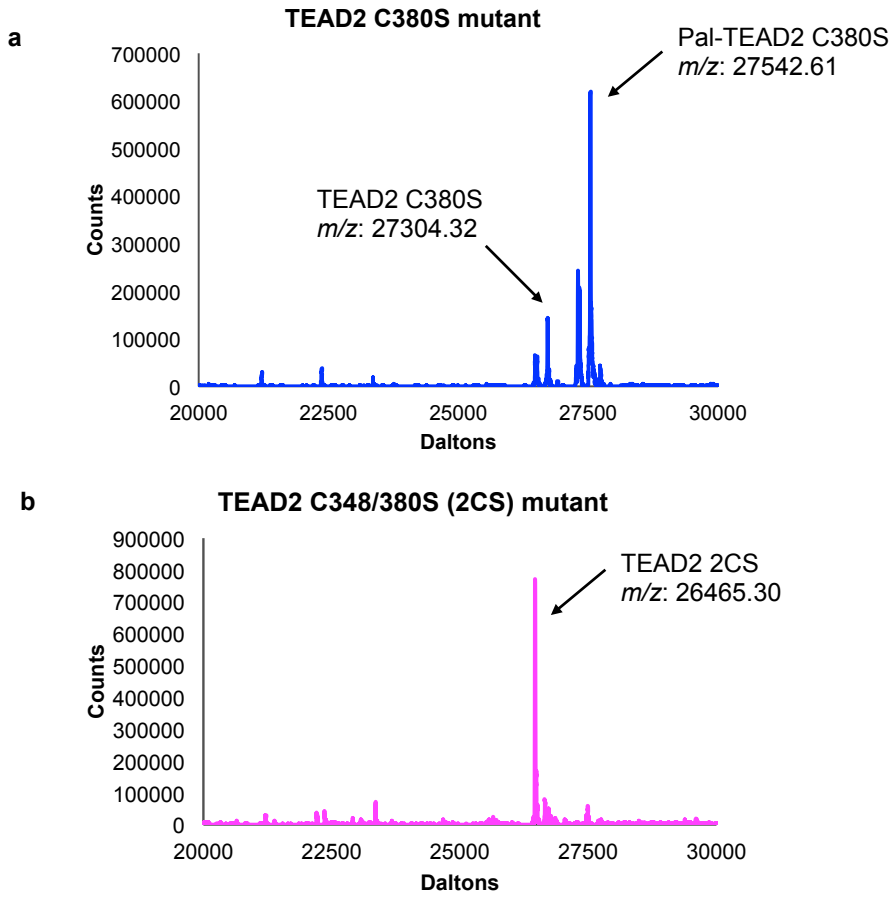


Supplementary Figure 3. Autopalmitoylation of TEAD2.
(a) Mass spectrometry analysis confirmed autopalmitoylation of recombinant TEAD2 YBD when incubated with palmitoyl-CoA *in vitro*.

(**b-c**) Time-dependent and palmitoyl-CoA dose-dependent autopalmitoylation of recombinant TEAD2 YBD *in vitro*.



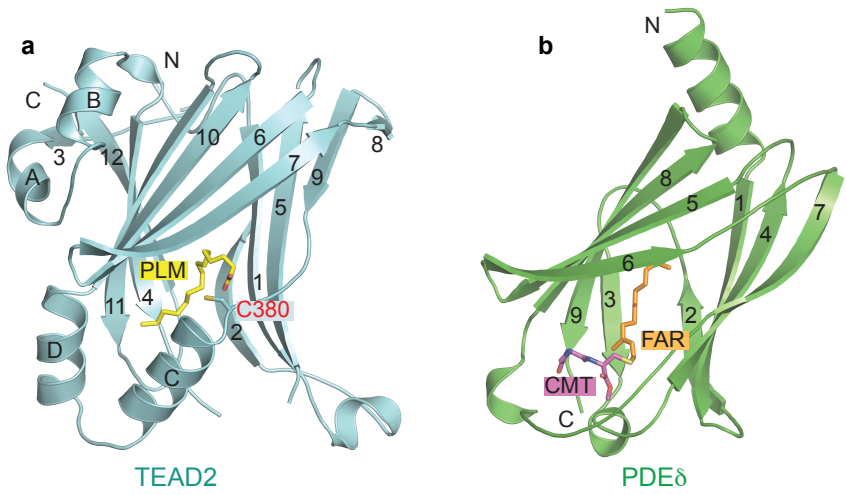
Supplementary Figure 4. The *Fo – Fc* omit electron density map for TEAD1-cyclic YAP (a) and mTEAD4–mYAP (b) in the deep hydrophobic pocket at the contour level of 3σ.
The electron density has shown a lipid-like molecule binding to the pocket; however, the density is truncated, and cannot be accurately assigned to a palmitate without prior knowledge of TEAD palmitoylation. The electron density in 3JUA seems to be connected to C360 of TEAD4, implicating a possible covalent modification on this residue.



Supplementary Figure 5. Mass spectra of TEAD2 C380 and 2CS mutants after the autopalmitoylation reaction. His-TEAD2 YBD (C380 or 2CS mutant) was incubated with palmitoyl-CoA and then subjected to the intact mass detection using QTF mass spectrometer.

(**a**) TEAD2 C380 mutant can still be palmitoylated *in vitro*. The mass peak with m/z of 27304 indicates unmodified His6-tagged TEAD2 C380S protein (without the first Met), and m/z of 27542 indicates the palmitoylated His6-tagged-TEAD2 C380 protein.

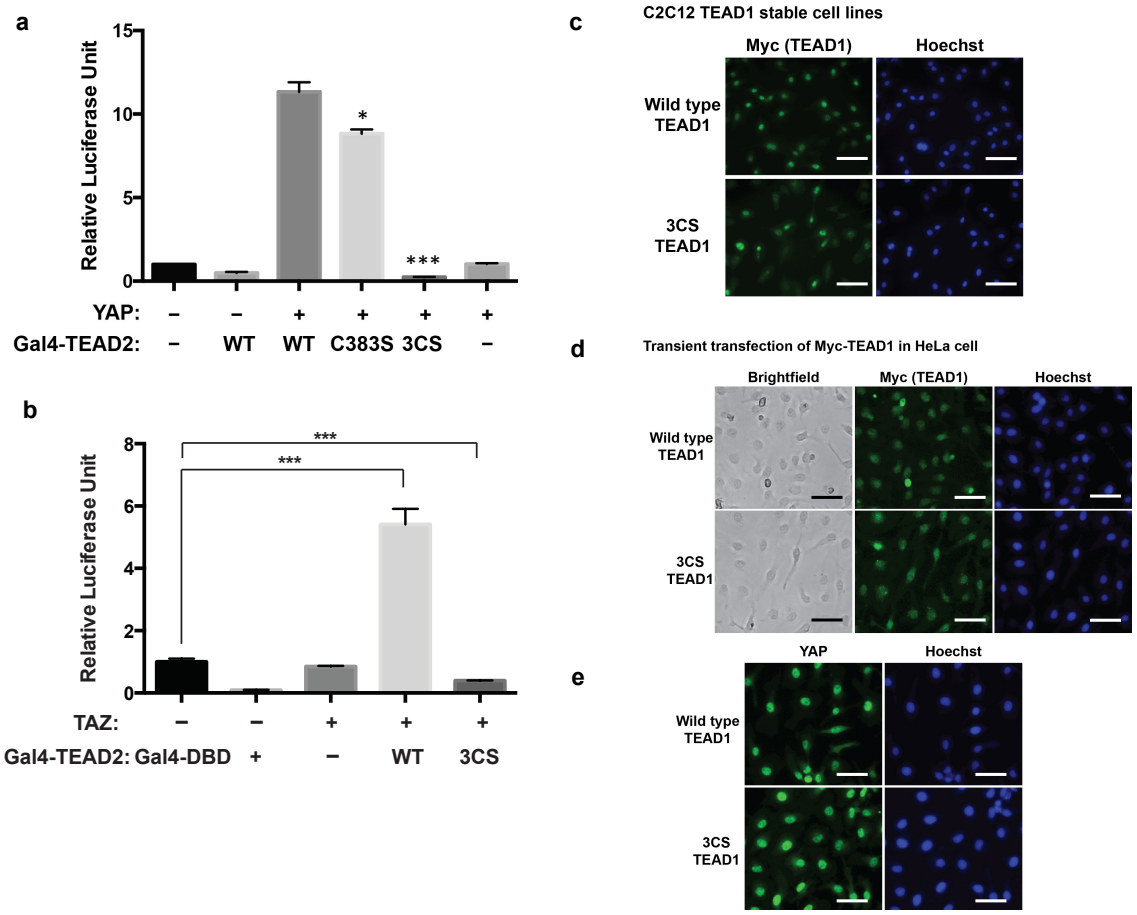
(**b**) TEAD2 2CS mutant cannot be palmitoylated *in vitro*. The mass peak with m/z of 27304 indicates unmodified His6-tagged TEAD2 C380S protein (without the first Met), and m/z of 26465.30 indicates the un-palmitoylated TEAD2 2CS protein (without the first Met and the C-terminal His tag). The His tag was cleaved during the mass spec analysis.



Supplementary Figure 6. Structural comparison between TEAD2 and PDEδ.

(a) Cartoon diagram of palmitate-bound TEAD2 YBD. TEAD2 is colored in cyan, and palmitate (PLM) is shown as yellow sticks.

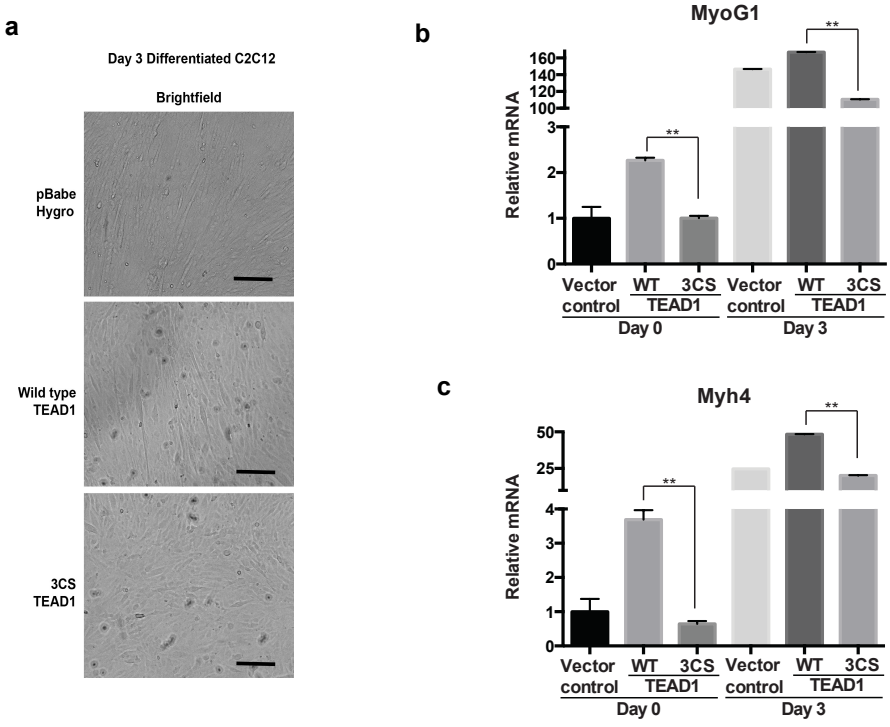
(**b**) Cartoon diagram of farnesylated PDEδ (PDB code: 3T5I). PDEδ is colored in green, and the farnesyl group (FAR) is shown as orange sticks and the linked C-terminal O-methylcysteine (CMT) is shown as magenta sticks.



Supplementary Figure 7. TEAD palmitoylation is required for its association to YAP/TAZ, and does not alter TEAD localization.

(**a-b**) Gal4-repsonsive luciferase assay of Gal4-TEAD2 and YAP interaction (**a**), and TAZ interaction (**b**). HEK293T cells were transfected with Gal4-UAS-Luc and the indicated plasmids. 48 h post transfection, cells were processed with Duo-Glo luciferase assay system (Promega) and luciferase activity was recorded using an EnVision plate reader (Perkin Elmer). A representative of three independent experiments is shown. (Data is shown as mean ± s.e.m., n=3, *P* values were determined using two-tailed t-tests. *, $p<0.01$, ***, $p<0.0001$)**.**

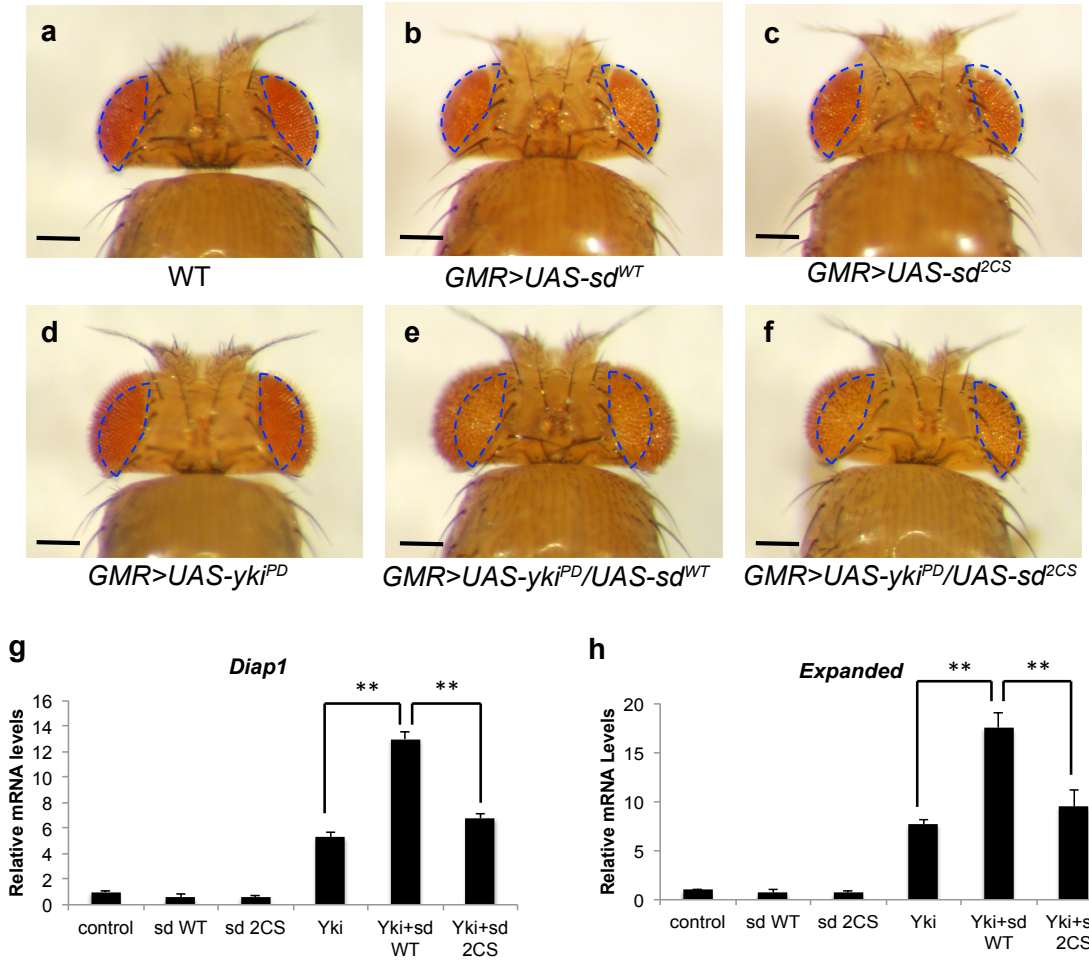
(**c-e**) Palmitoylation does not alter TEAD1 localization. Palmitoylation-deficient TEAD1 mutant (3CS) remains localized in the nucleus in C2C12 cells (**c**) and HeLa cells (**d**), and it does not alter YAP localization (**e**). Cellular localization of endogenous YAP was visualized by immunostaining and images were captured using Nikon Digital Insight microscope. Scale bar: 100 μm.



Supplementary Figure 8. Palmitoylation-deficient mutant of TEAD1 inhibited TEAD target genes expression in C2C12 cells.

(**a**) Bright field images of cells under differentiation conditions for 3 days. Scale bar: 100 μm.

(**b**-**c**) TEAD 3CS mutant blocked the expression of myogenic markers *MyoG1* (**b**) and *Myh4* (**c**) in C2C12 cell. RNA samples of C2C12 stably expressed vector control, wild type and 3CS mutant of TEAD1 were collected and cDNA of each were synthesized. mRNA levels of each gene were determined by qRT-PCR using SYBR Green and normalized to *GAPDH*. (Data are represented as mean ± SEM, n=3. *P* values were determined using two-tailed t-tests. **, $p<0.01$)



Supplementary Figure 9. Palmitoylation-deficient mutant of Scalloped (Sd) impaired Yki-PD induced eye growth.

Top view of compound eyes from the following genotypes: (**a**) *GMR-gal4/+*, (**b**) *GMR-gal4/UAS-sd^{WT}*, (**c**) *GMR-gal4/UAS-sd^{2CS}*, (**d**) *GMR-gal4, UAS-yki^{PD}*, (**e**) *GMR-gal4, UAS-yki^{PD}/UAS-sd^{WT}*, (**f**) *GMR-gal4, UAS-yki^{PD}/UAS-sd^{2CS}*. The images were taken with the same magnification. The size of the eye in wild type control flies is marked in blue dashed line, and the same area is shown in all images to facilitate comparison. Scale bar: 150 µm.

(**g-h**) Yki target gene expression (Diap1 and Expanded) in fly S2 cells transfected with indicated constructs by qRT-PCR. (Data are represented as mean ±, SEM, n=3. *P* values were determined using two-tailed t-tests. **, p<0.01)

Figure 1c - Input

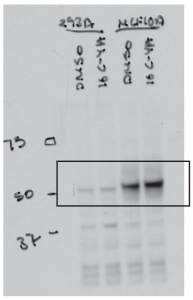


Figure 1c - Streptavidin pull-down

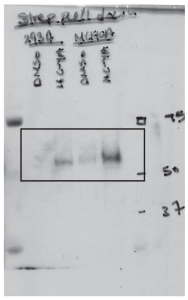


Figure 1c - TEAD2

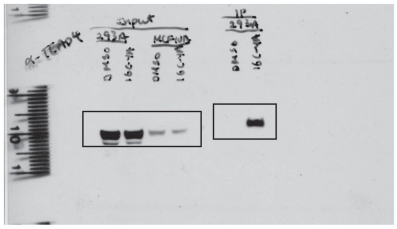


Figure 1c - TEAD4

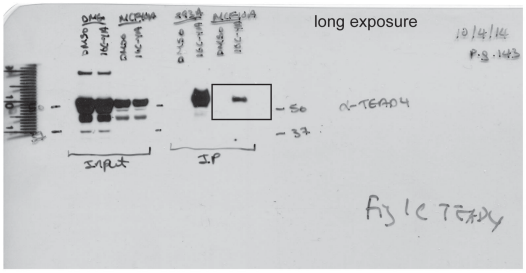
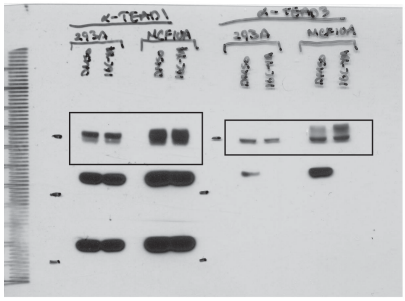


Figure 1c - TEAD1 Input

Figure 1c - TEAD3 Input



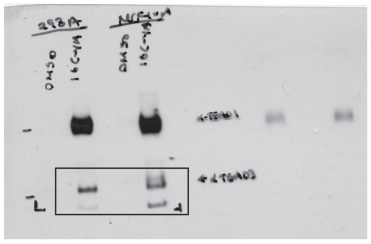


Figure 1c - TEAD3 Strep pull-down

long exposure

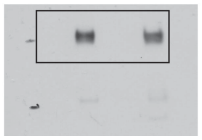
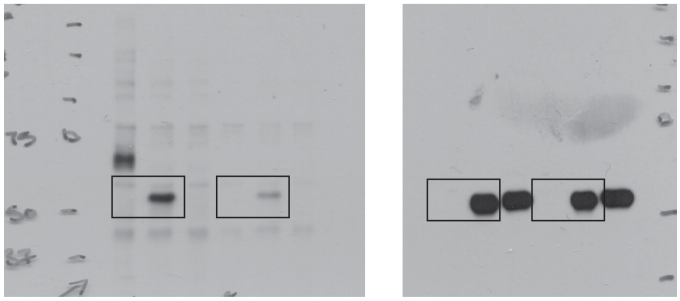


Figure 1c - TEAD1 Strep pull down

short exposure

Figure 1d



Supplementary Figure 10. Full images of the blots in Figure 1c and 1d.

Figure 2a

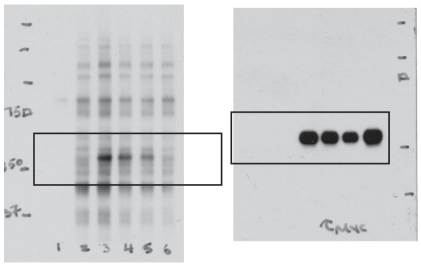


Figure 2b (5.23.14)

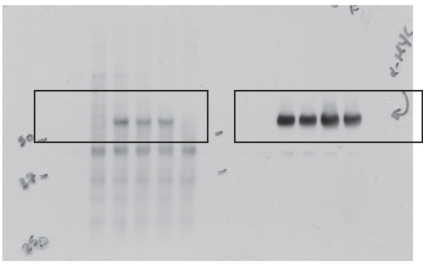
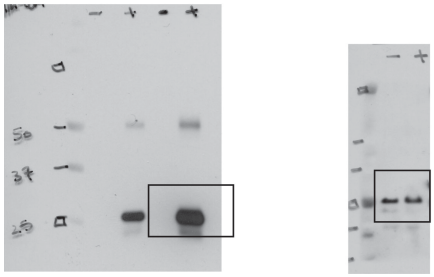
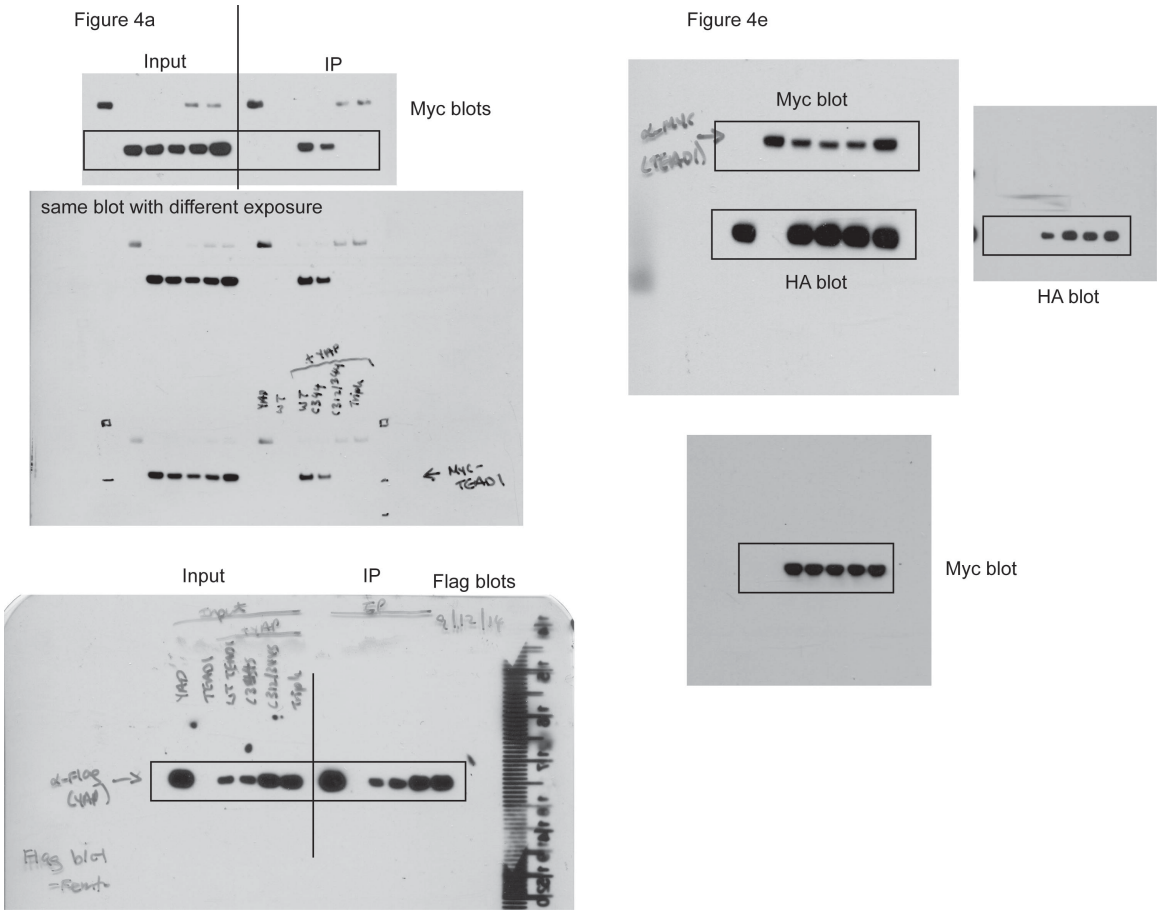


Figure 2c (1.22.15)

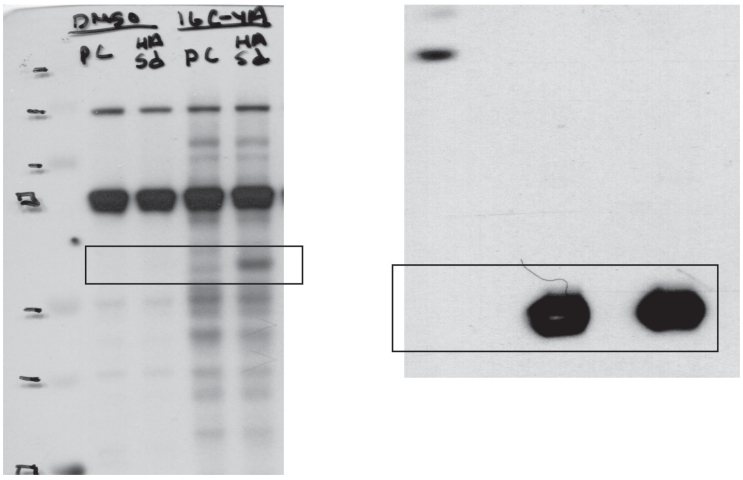


Supplementary Figure 11. Full images of the blots in Figure 2a, 2b and 2c.



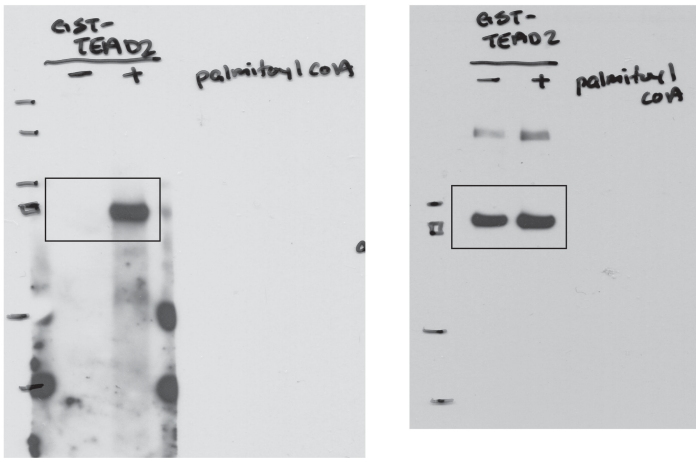
Supplementary Figure 12. Full images of the blots in Figure 4a and 4e.

Supplementary Figure 1b

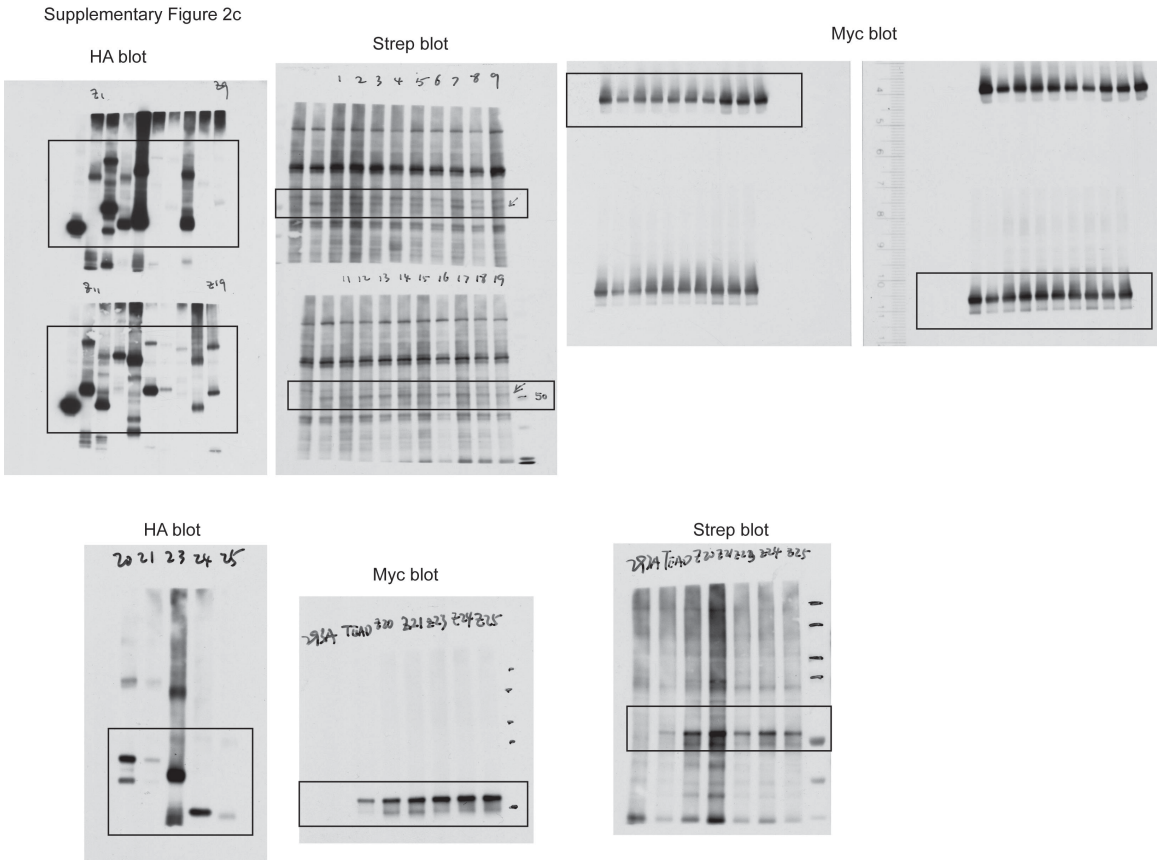


Supplementary Figure 13. Full images of the blots in Supplementary Figure 1b.

Supplementary Figure 2b



Supplementary Figure 14. Full images of the blots in Supplementary Figure 2b.



Supplementary Figure 15. Full images of the blots in Supplementary Figure 2c.

Reference

1 Zheng, B. *et al.* 2-Bromopalmitate analogues as activity-based probes to explore palmitoyl acyltransferases. *J Am Chem Soc* **135**, 7082-7085, doi:10.1021/ja311416v (2013).