Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supplementary Information

A covalent inhibitor of the YAP-TEAD transcriptional complex identified by high-throughput screening.

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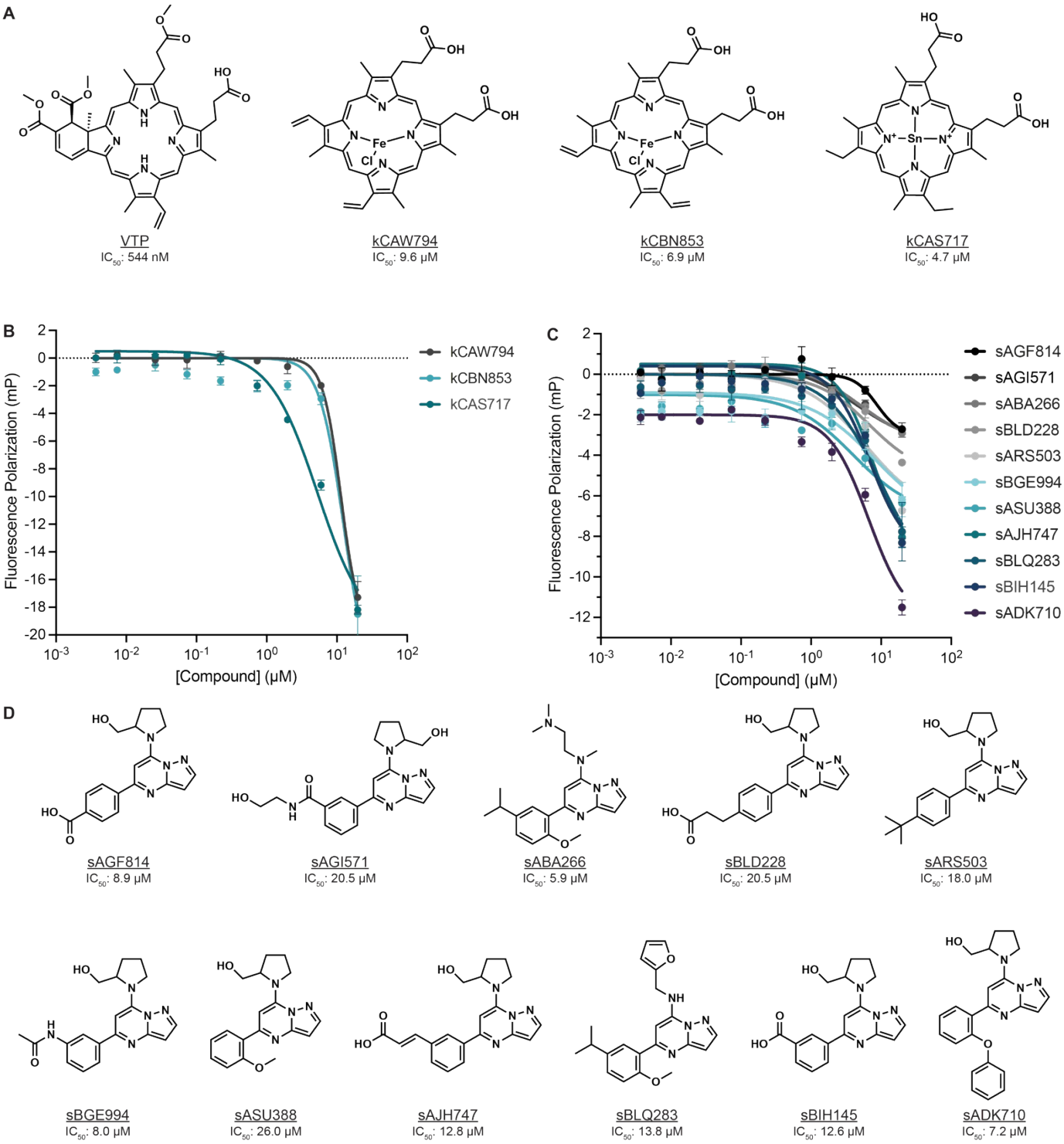

Figure S1. Additional identified compounds from HTS. A) Structures of verteporfin (VTP) and porphyrin containing molecules identified from the screen along with their respective IC_{50} values in the primary FP screening assay. B) Fluorescence polarization values in response to the indicated concentrations of the identified porphyrin containing compounds identified from HTS (n=3, mean ± SEM). C) Fluorescence polarization values in response to the indicated concentrations of the identified pyrazolopyrimidine containing compounds (n=3, mean ± SEM). D) Structures of pyrazolopyrimidine molecules and their respective IC_{50} values in the primary FP screening assay.

Batch	Plates	Z’	CV	Hit Rate	Confirmation Rate	RBZ	Confirmed Hits
1	63	0.68	3.2	1.55%	-	≤ -3	-
2	89	0.68	3.9	0.98%	6.85%	≤ -4	86
3	6	0.71	5.1	-	1.83%	≤ -3	19
4	83	0.63	6.3	1.16%	-	≤ -3	-
5	83	0.72	5.3	2.47%	4.4%	≤ -3	50
6	81	0.78	6.8	1.22%	-	≤ -3	-
7	24	0.71	3.5	0.91%	4.2%	≤ -3	90
8	63	0.68	4.4	1.69%	-	≤ -3	-
9	78	0.67	3.6	1.46%	1.4%	≤ -3	22
10	100	0.67	6.7	1.07%	3.7%	≤ -3	53
11	45	0.65	5.6	1.5%	-	≤ -3	-
12	44	0.71	8	-	1.6%	≤ -3	288
13	7	0.67	6.5	-	-	-	-

Supplementary Table 1. High throughput screening summary. Statistics for each of the screening batches. (RBZ, robust Z score).

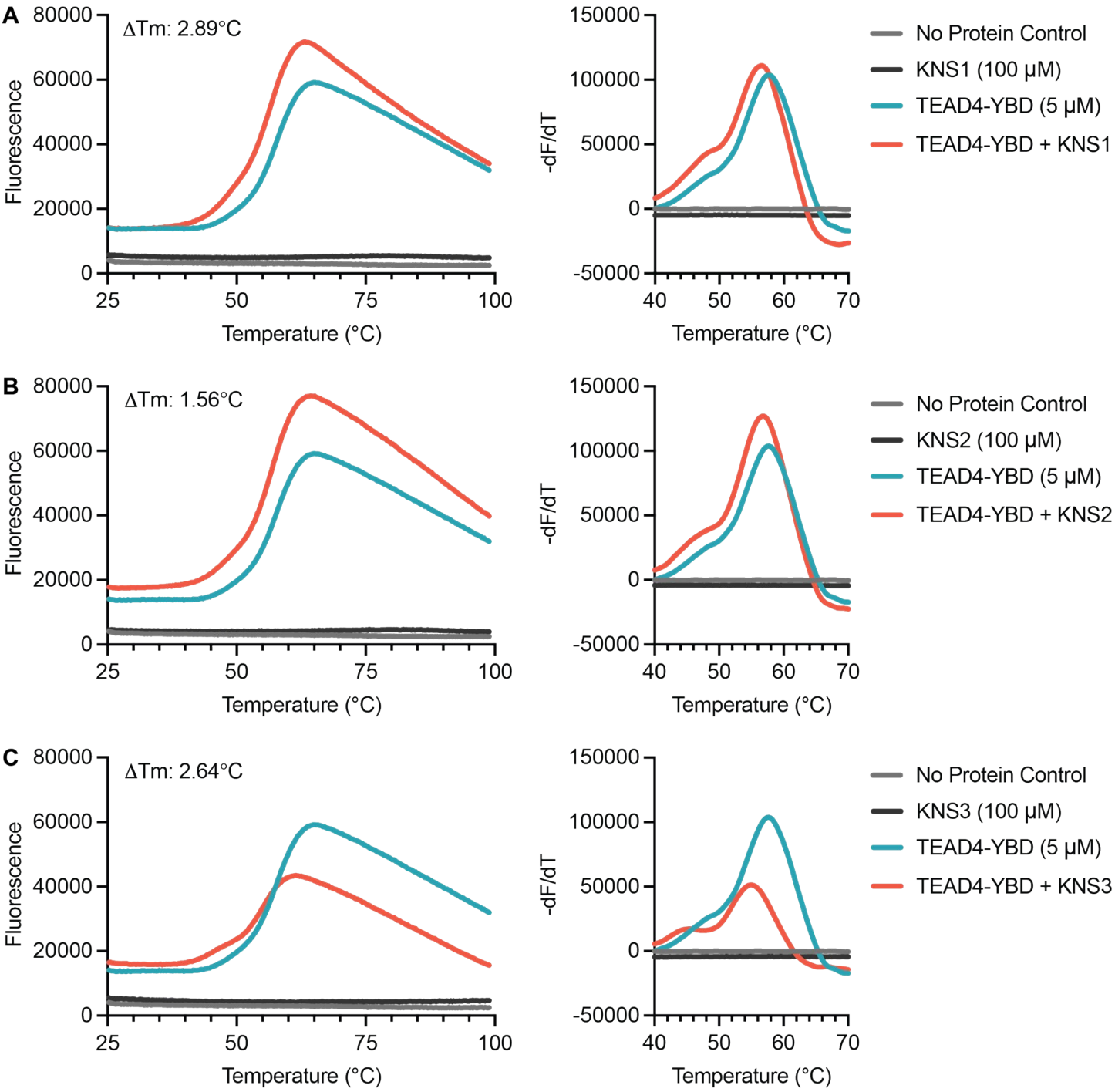

Figure S2. Identified inhibitors bind TEAD4. Fluorescence values (RFU) and their derivatives (-dF/dT) obtained from a thermal denaturation assay in which TEAD4-YBD was exposed to KNS1 (A), KNS2 (B), or KNS3 (C) (n=5, mean).

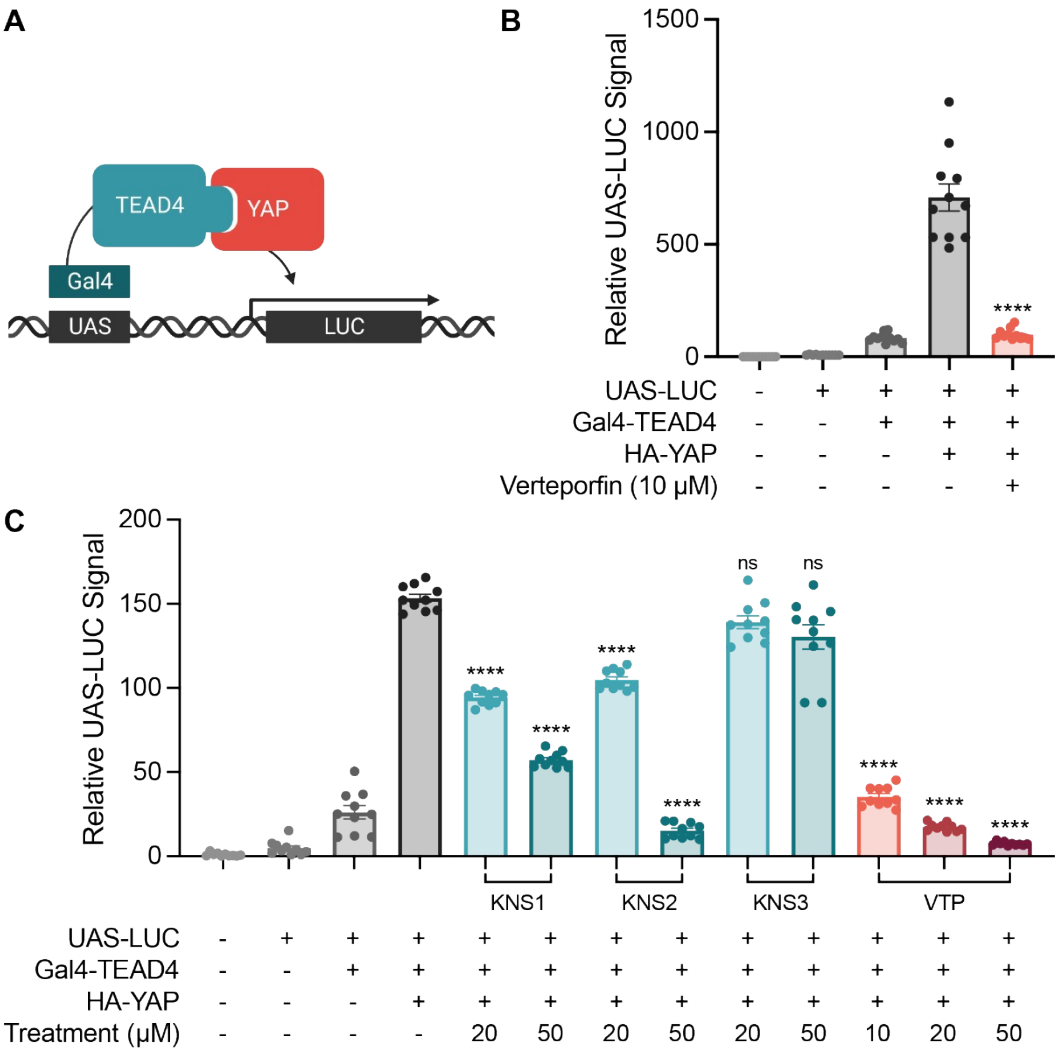

Figure S3. Identified inhibitors inhibit TEAD:YAP transcriptional activation. A) Schematic depicting Gal4 assay. B) Relative UAS-LUC driven luminance signal from HEK293T cells transfected with the indicated plasmids and then treated with VTP (10 μM) for 24 hours (n=11, mean ± SEM). C) Relative UAS-LUC driven luminance signal from HEK293T cells transfected with the indicated plasmids and then treated with the indicated compounds for 24 hours (n=10, mean ± SEM). Significance was determined by Welch and Brown-Forsythe ANOVA; ns = not statistically significant, ****p<0.0001.

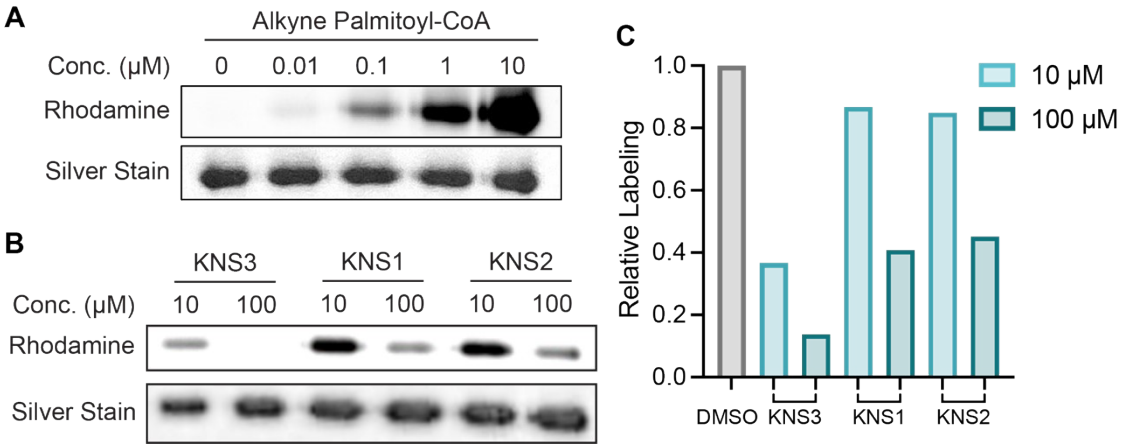

Figure S4. Identified inhibitors prevent lipidation of TEAD4. (A) Fluorescent scans of rhodamine labeling post click reaction after exposure of TEAD4-YBD to alkyne palmitoyl-CoA at the indicated concentrations. (B) Fluorescent scans of rhodamine labeling (B) and densitometry-based quantification (C) post click reaction after exposure of TEAD4-YBD to the indicated concentrations of compound followed by labeling with alkyne palmitoyl-CoA (100 nM; normalized to fluorescent intensity measured for DMSO treatment).

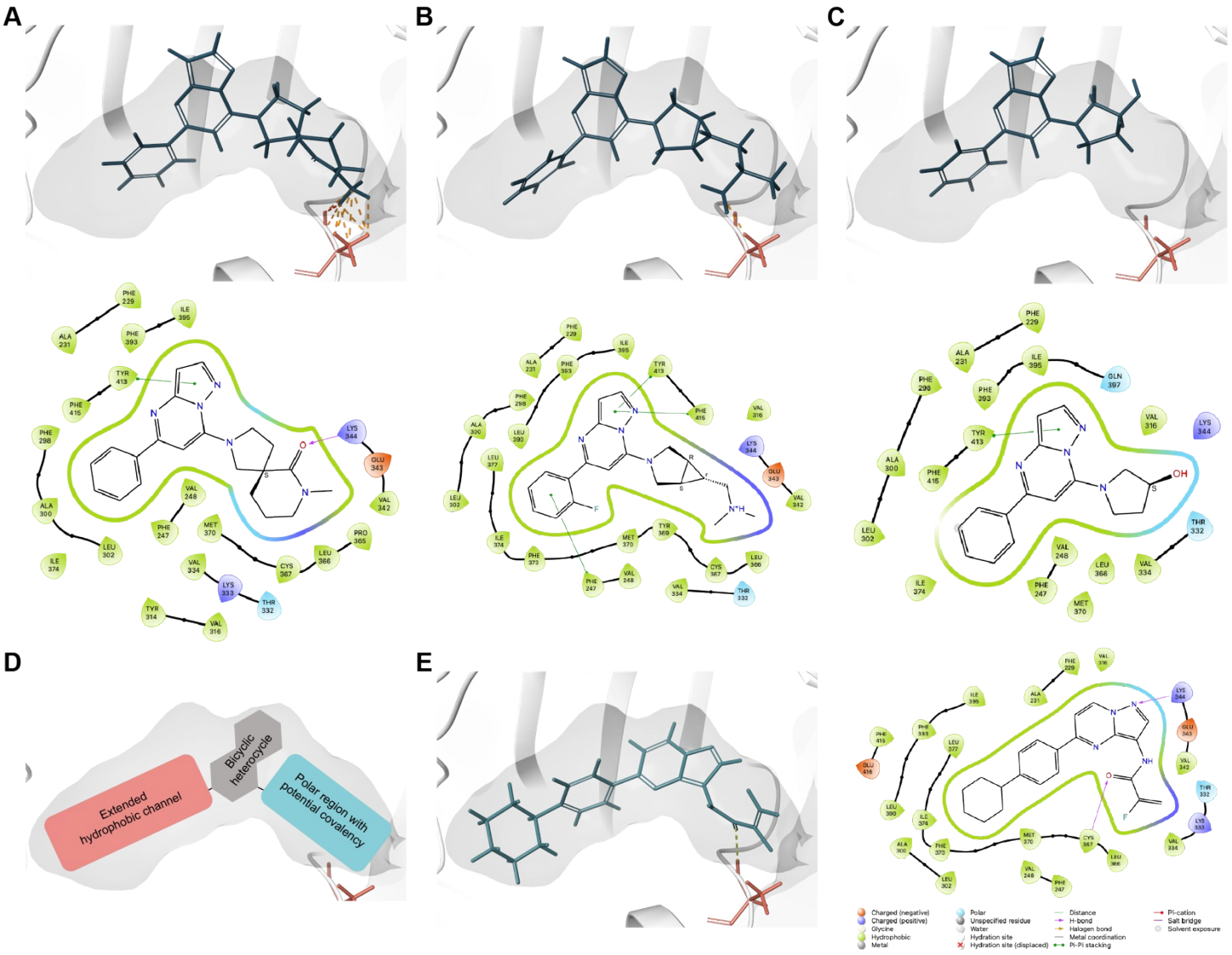

Figure S5. Identified inhibitors bind the TEAD palmitoylation site in a similar binding pose. Top docking poses of KNS1 (A), KNS2 (B), and KNS3 (C) in the palmitoylation pocket of TEAD4 (PDB: 5OAQ). Conserved cysteine 367 is depicted in in red. Ligand interaction map is provided below each binding pose. D) Schematic depicting a conserved interaction roadmap in the design of TEAD inhibitors like mCMY020. E) Top docking pose of mCMY020 in the palmitoylation pocket of TEAD4 (PDB: 5OAQ). Ligand interaction diagram and legend are provided on the right.

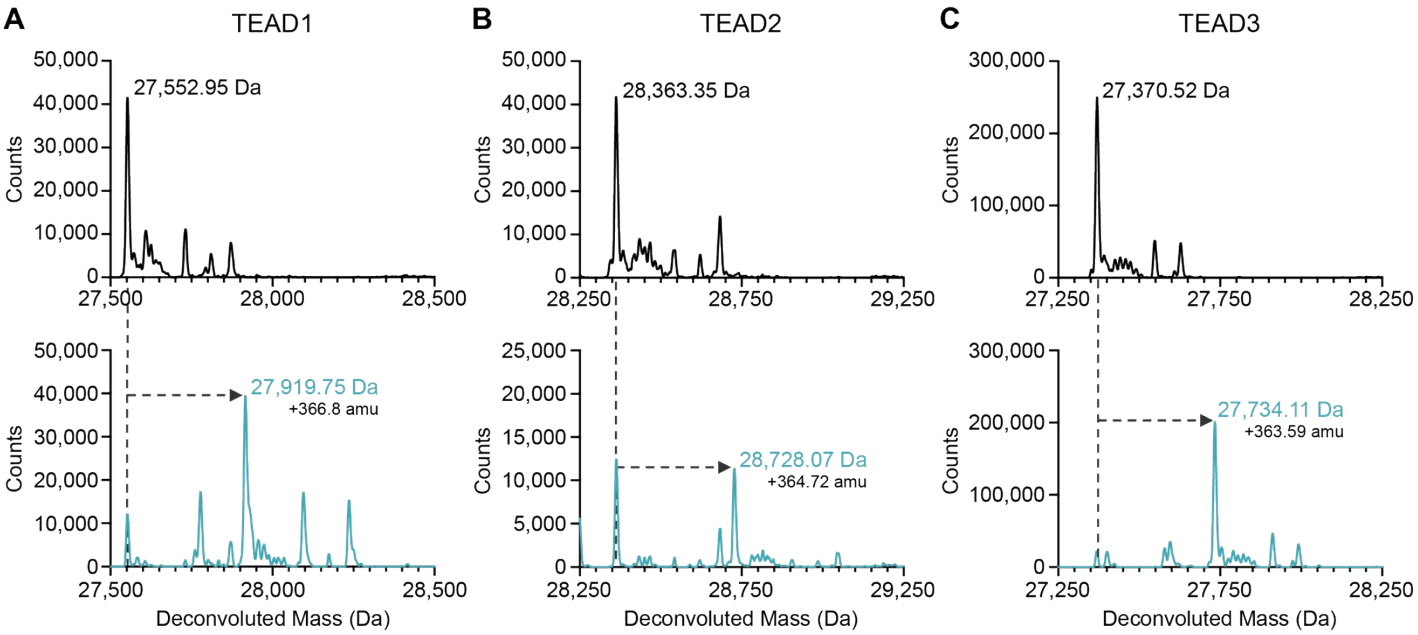

Figure S6. mCMY020 covalently binds to TEADs. TEAD1 (A), TEAD2 (B), and TEAD3 (C) in the presence (blue, bottom) or absence (black, top) of mCMY020 analyzed by ESI-TOF mass spectrometry.

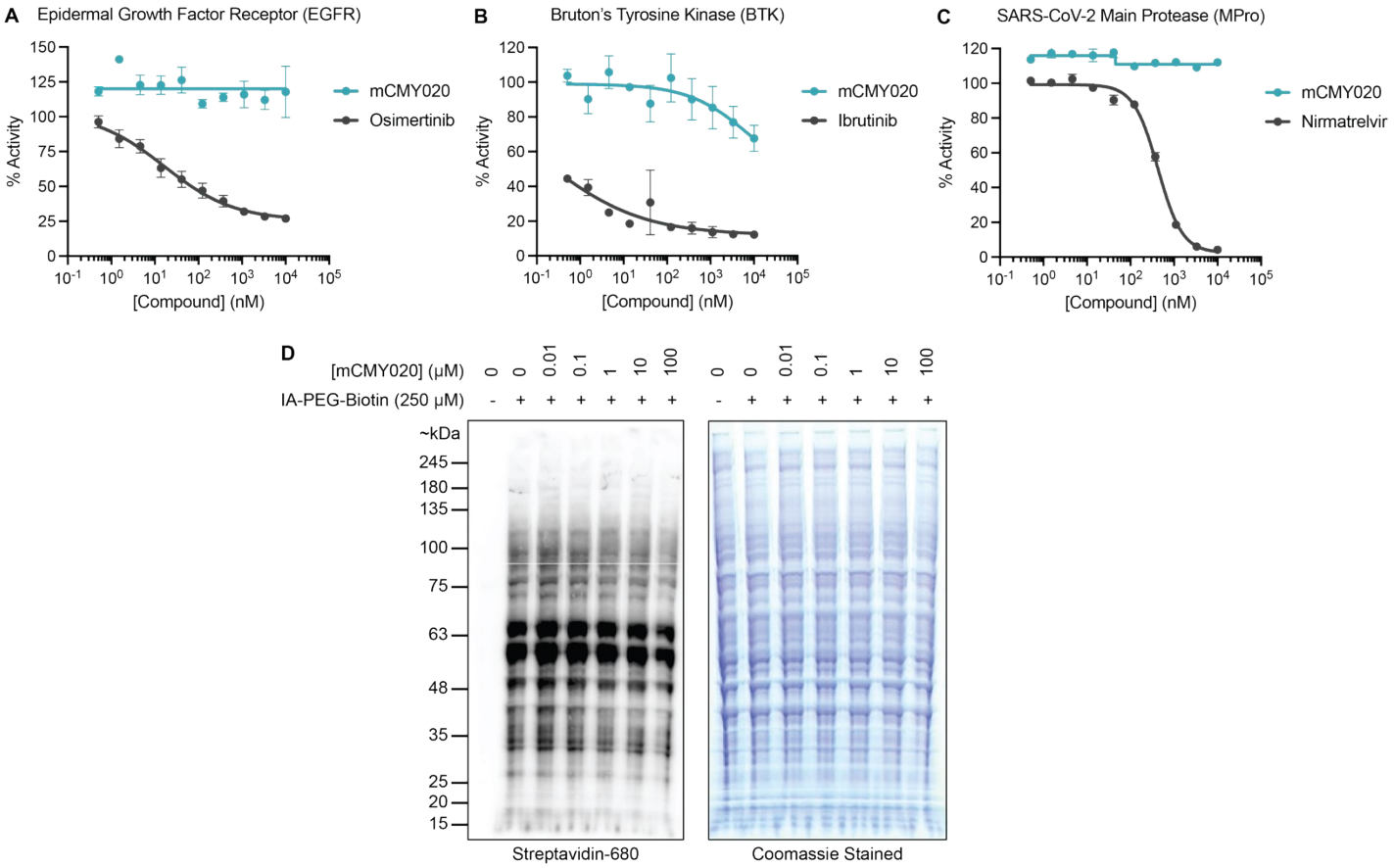

Figure S7. mCMY020 is selective for TEADs. Percent activity of (A) EGFR, (B) BTK, and (C) MPro treated with the indicated concentrations of mCMY020 and their known covalent inhibitors. (D) Representative western blot (left) and Coomassie stain (right) of iodoacetamide labeled proteins in NCI-H226 cells after pre-treatment with the indicated concentrations of mCMY020.

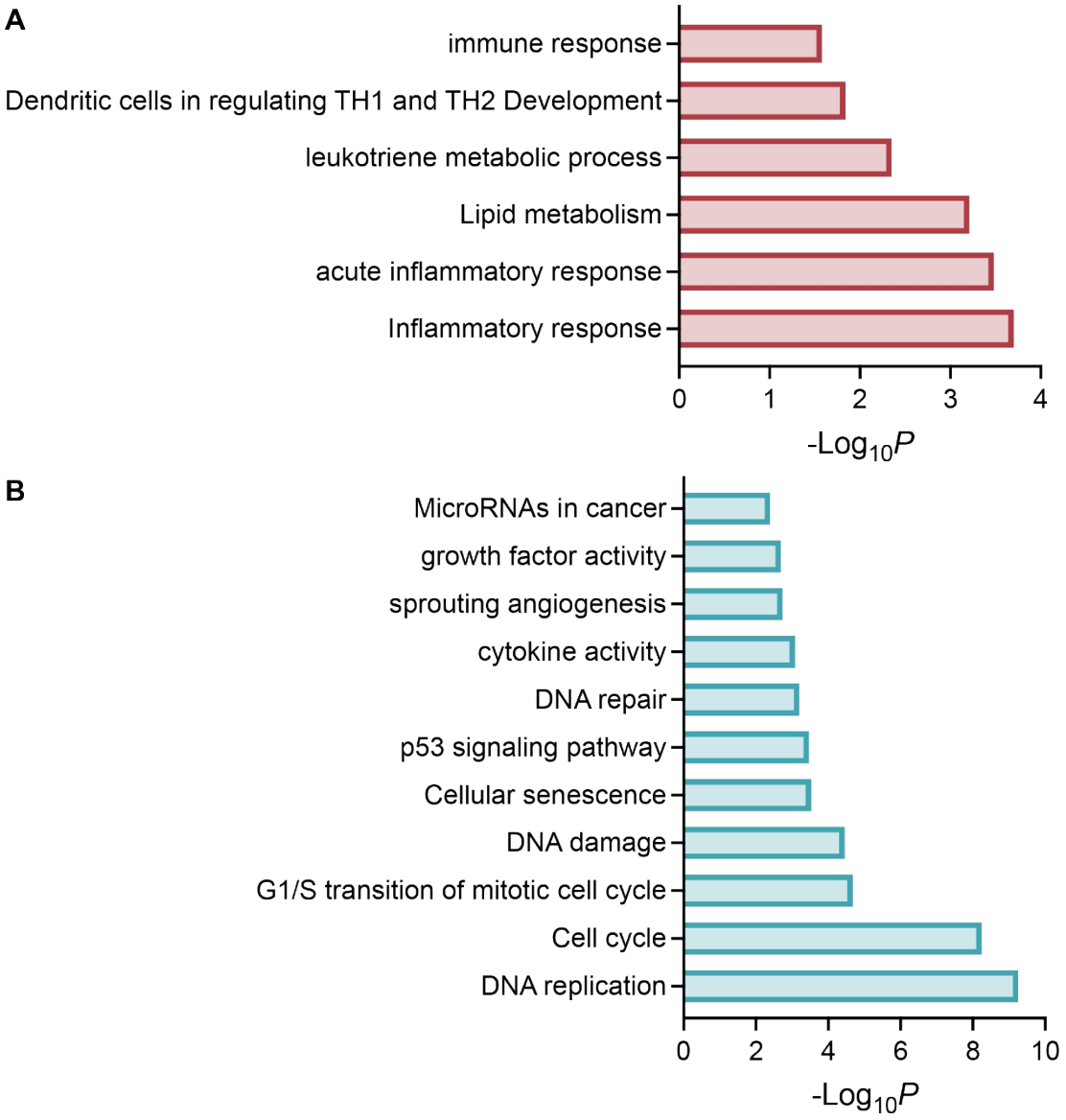

Figure S8. Gene ontology (GO) enrichment analysis. -Log$_{10}$*P* values of representative up-regulated (A) and down-regulated (B) gene sets derived from Gene ontology (GO) enrichment analysis of RNA-sequencing data obtained from NCI-H226 cells treated for 24 hours with 10 μM mCMY020.