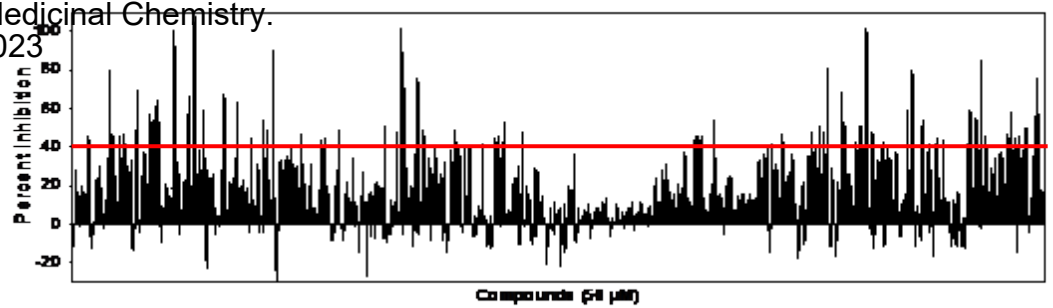


Figure S1. A total 658 chloroacetamide fragments and compounds were screened to identify TEAD4•YAP1 interaction inhibitors. TEAD4 (64 nM) was incubated with fragments and compounds at 50 µM concentration for 24 h at 4°C prior to binding detection by polarization using fluorescently labeled $YAP1_{60-99}$ peptide (n=2). The red line was drawn to highlight the 40% threshold that was used to select candidates for follow-up studies.

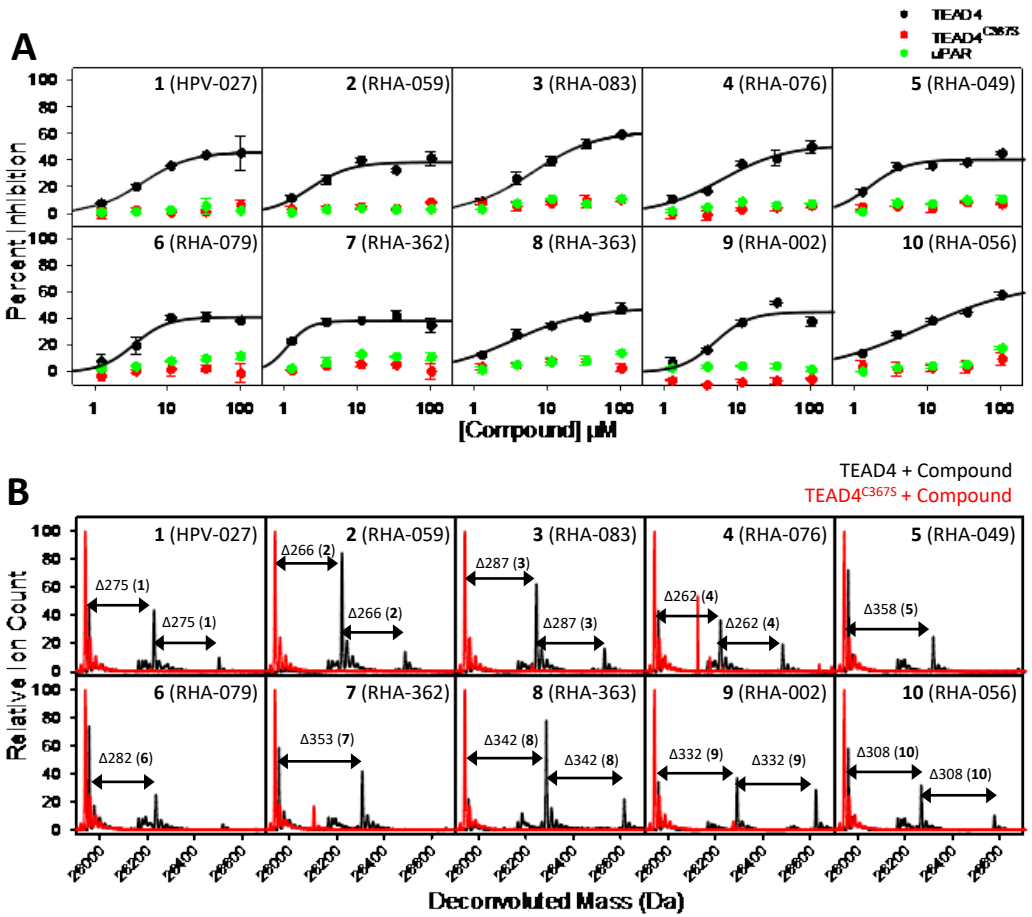


Figure S2. (A) Inhibition of TEAD4, TEAD4^{C367S} and uPAR interactions by compounds using our FP assays after incubation of protein with compound for 24 h at 4°C (TEAD4 n=3; TEAD4^{C367S} and uPAR n=2) **(B)** Whole-protein mass spectrometry of TEAD4 and TEAD4^{C367S} mutant after 24 h 4 °C incubation with 100 µM compound. TEAD4 was detected at 25953 Da, TEAD4^{C367S} was detected at 25937 Da, and peaks for protein-compound adducts were also detected with a mass that matches the covalent complex following reaction of chloroacetamide with central pocket Cys-367 cysteine. In some cases, an additional peak was detected that corresponds to the protein in a covalent complex with two compounds likely due to reaction at another cysteine on the protein. These are depicted by the second peak in the red spectrum in each panel.

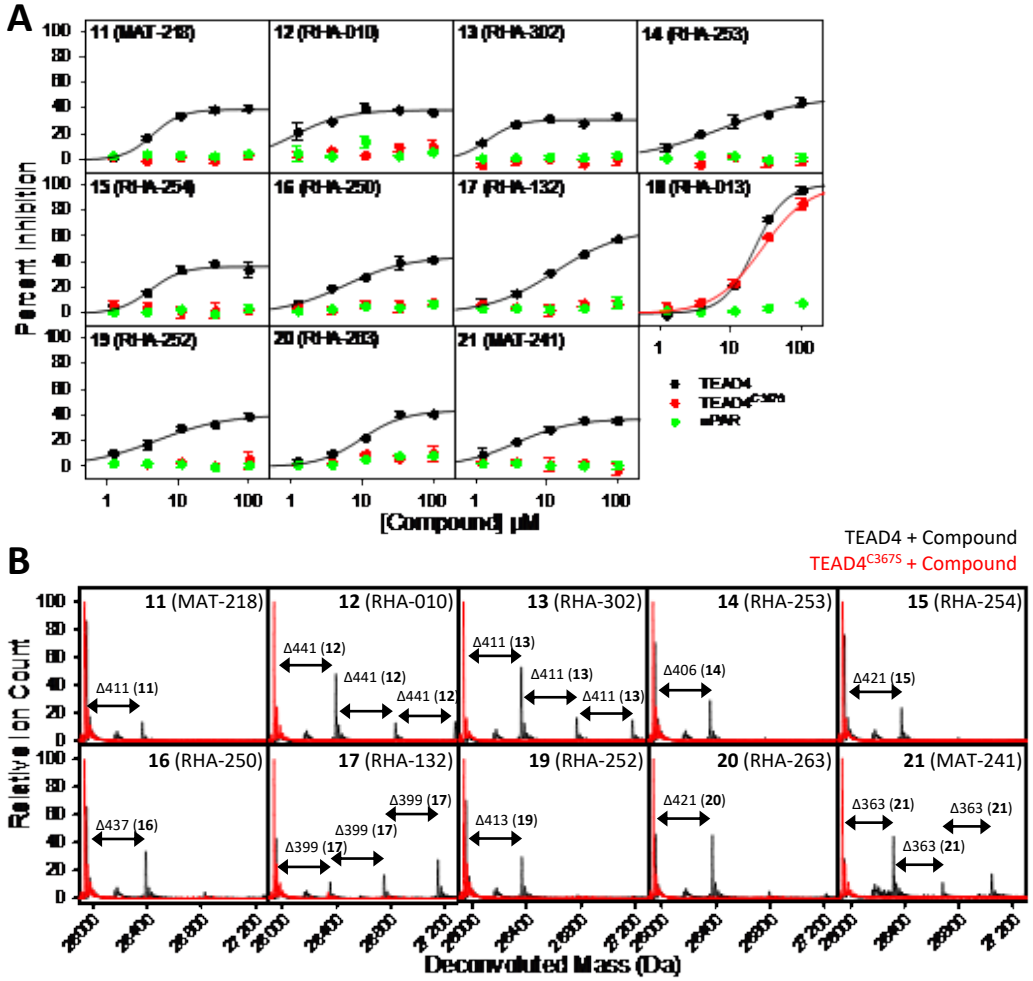


Figure S3. (A) Inhibition of TEAD4, TEAD4^{C367S} and uPAR interactions by compounds using our FP assays after incubation of protein with compound for 24 h at 4°C (TEAD4 n=3; TEAD4^{C367S} and uPAR n=2). **(B)** Whole-protein mass spectrometry of TEAD4 and TEAD4^{C367S} mutant after 24 h 4 °C incubation with 100 µM compound. TEAD4 was detected at 25953 Da, TEAD4^{C367S} was detected at 25937 Da, and peaks for protein-compound adducts were also detected with a mass that matches the covalent complex following reaction of chloroacetamide with central pocket Cys-367 cysteine. In some cases, additional peaks were detected that correspond to the protein in a covalent complex with two or three compounds likely due to reaction at another cysteine on the protein. These are depicted by the second peak in the red spectrum in each panel.

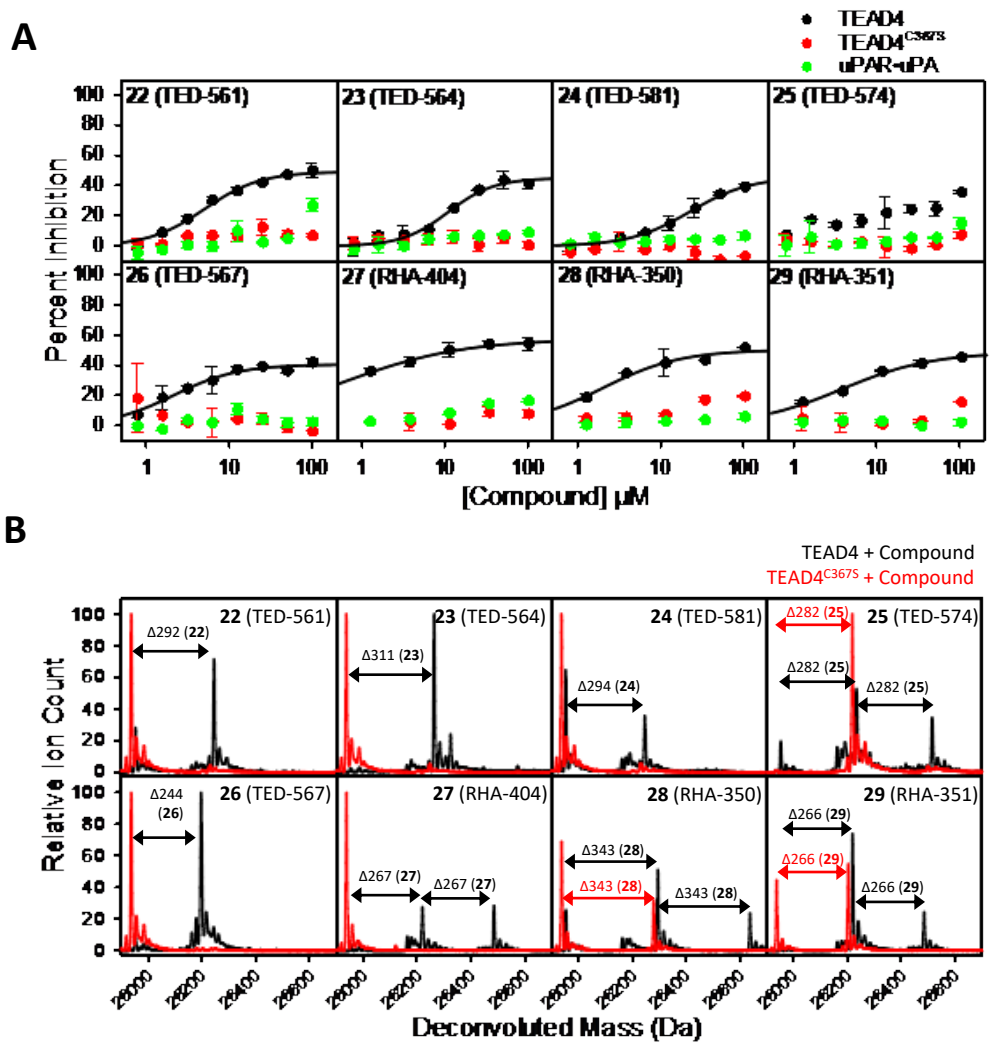
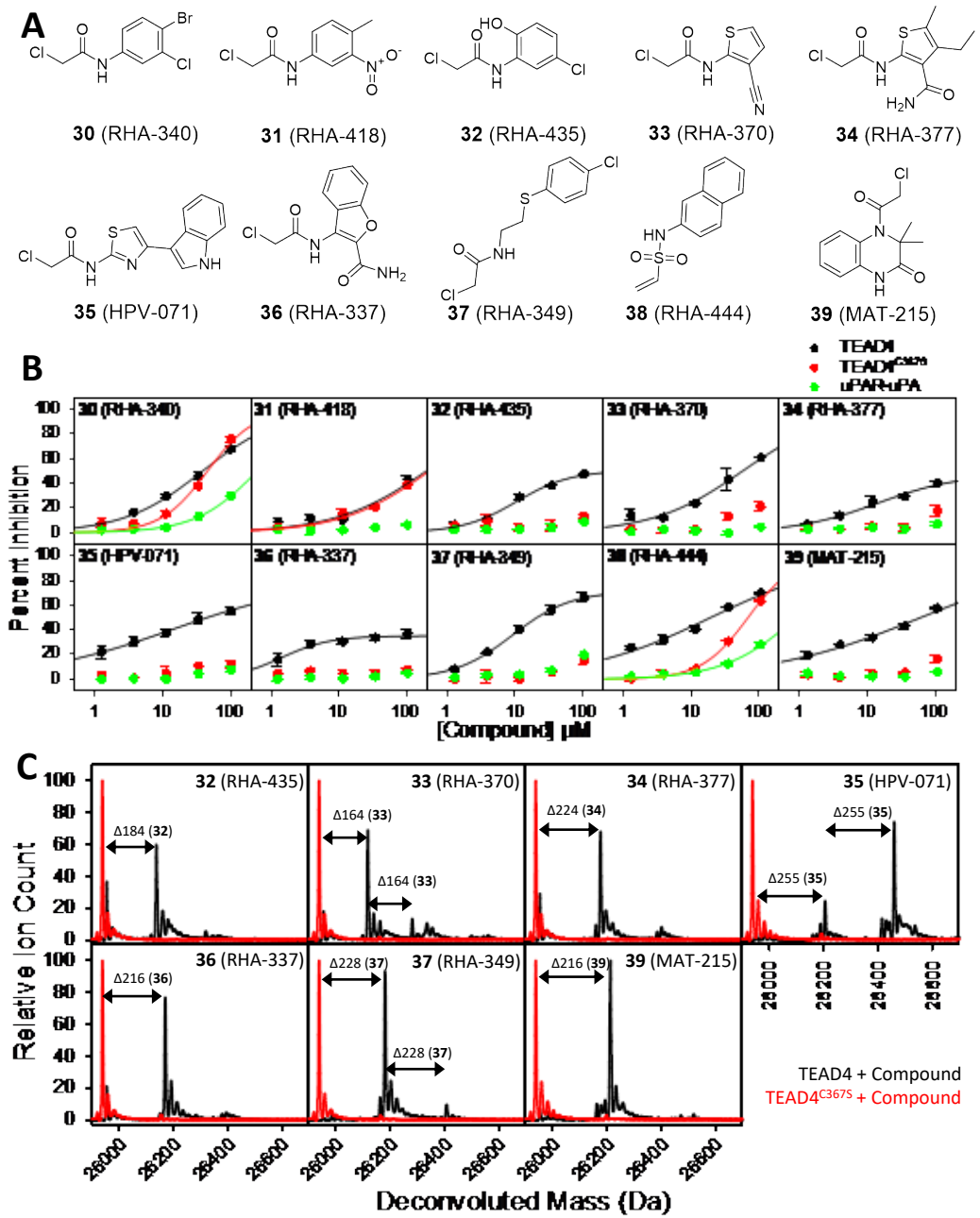
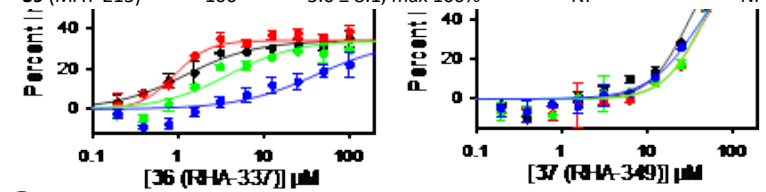


Figure S4. (A) Inhibition of TEAD4, TEAD4^{C367S} and uPAR interactions by compounds using our FP assays after incubation of protein with compound for 24 h at 4°C (TEAD4 n=3; TEAD4^{C367S} and uPAR n=2). **(B)** Whole-protein mass spectrometry of TEAD4 and TEAD4^{C367S} mutant after 24 h 4 °C incubation with 100 µM compound. TEAD4 was detected at 25953 Da, TEAD4^{C367S} was detected at 25937 Da, and peaks for protein-compound adducts were also detected with a mass that matches the covalent complex following reaction of chloroacetamide with central pocket Cys-367 cysteine. In some cases, an additional peak was detected that corresponds to the protein in a covalent complex with two compounds likely due to reaction at another cysteine on the protein. These are depicted by the second peak in the red spectrum in each panel.


A
30 (RHA-340)
31 (RHA-418)
32 (RHA-435)
33 (RHA-370)
34 (RHA-377)
35 (HPV-071)
36 (RHA-337)
37 (RHA-349)
38 (RHA-444)
39 (MAT-215)
B
TEAD4
TEAD4C367S
uPAR-uPA
Percent Inhibition
[Compound] µM
C
Relative Ion Count
Δ184 (32)
Δ164 (33)
Δ224 (34)
Δ255 (35)
Δ216 (36)
Δ228 (37)
Δ216 (39)
Deconvoluted Mass (Da)
TEAD4 + Compound
TEAD4C367S + Compound

F

Compound	TEAD4 Mass Adduct (%)	TEAD4 WT IC_{50} at 24 h (μM)	TEAD4^{C367S} IC_{50} at 24 h (μM)	uPAR•AE-147 IC_{50} at 24 h (μM)	LipE
30 (RHA-340)	ND	38.0 ± 3.8, max 100%	44.8 ± 3.1	242 ± 67.5	0.9
31 (RHA-418)	ND	170 ± 60.1, max 100%	207 ± 57.5	NI	1.3
32 (RHA-435)	61	10.3 ± 3.8, max 49%	NI	NI	2.6
33 (RHA-370)	70, 12	67.4 ± 12.1, max 100%	NI	NI	3.3
34 (RHA-377)	68	13.0 ± 9.4, max 48%	NI	NI	3.0
35 (HPV-071)	23, 77	3.5 ± 0.5, max 60%	NI	NI	2.2
36 (RHA-337)	79	1.4 ± 0.3, max 35%	NI	NI	4.4
37 (RHA-349)	91, 9	8.3 ± 0.8, max 73%	NI	NI	2.1
38 (RHA-444)	ND	20.9 ± 14.8, max 100%	50.2 ± 20.4	285 ± 183	2.2
39 (MAT-215)	100	5.6 ± 8.1, max 100%	NI	NI	3.8



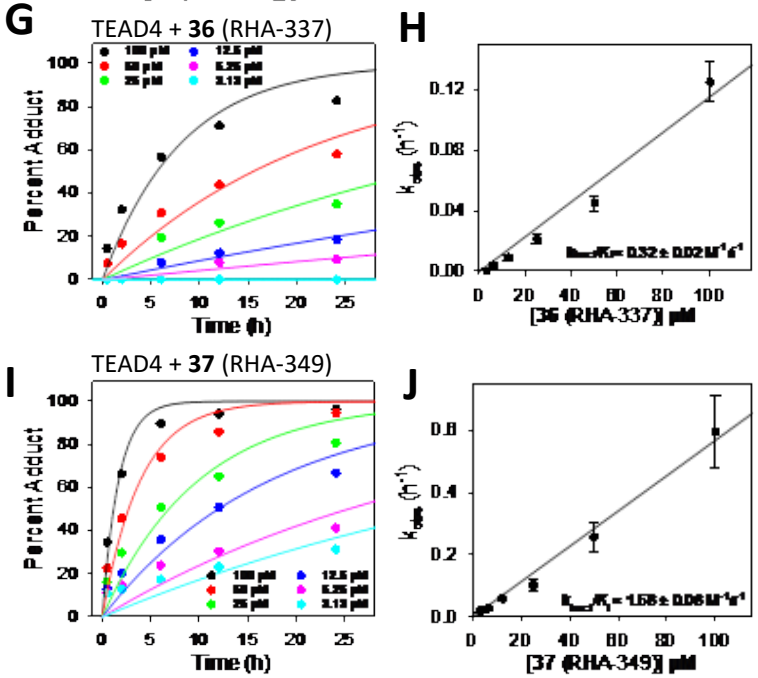


Figure S5. (A) Chemical structure of ten fragments that inhibit the TEAD4•YAP1 interaction. **(B)** Inhibition of TEAD4, TEAD4^{C367S} and uPAR interactions by compounds using our FP assays after incubation of protein with compound for 24 h at 4°C (TEAD4 n=3; TEAD4^{C367S} and uPAR n=2). **(C)** Whole-protein mass

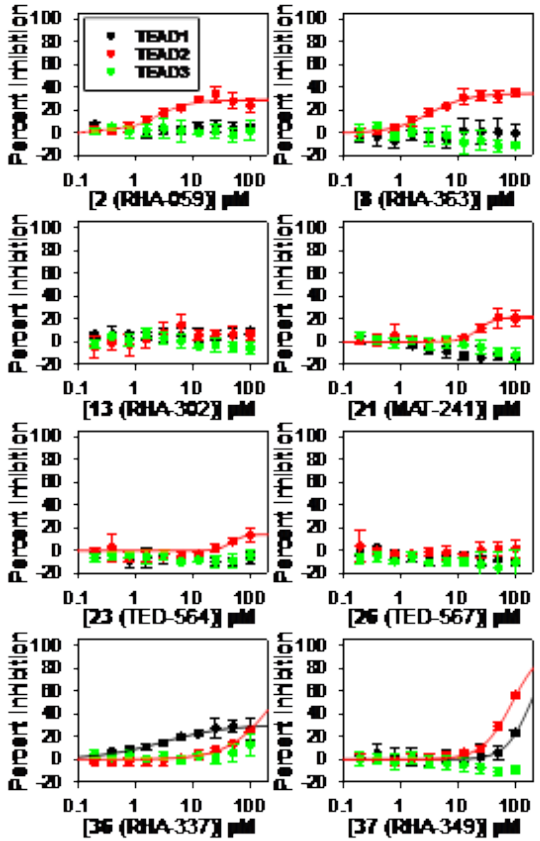


Figure S6. Inhibition of TEAD1-3 binding to fluorescently labeled YAP1 peptide**.** TEAD1-3 were incubated with varying concentrations of compounds for 24 h at 4°C prior to detection of binding to fluorescently labeled $YAP1_{60-99}$ peptide (n=3).

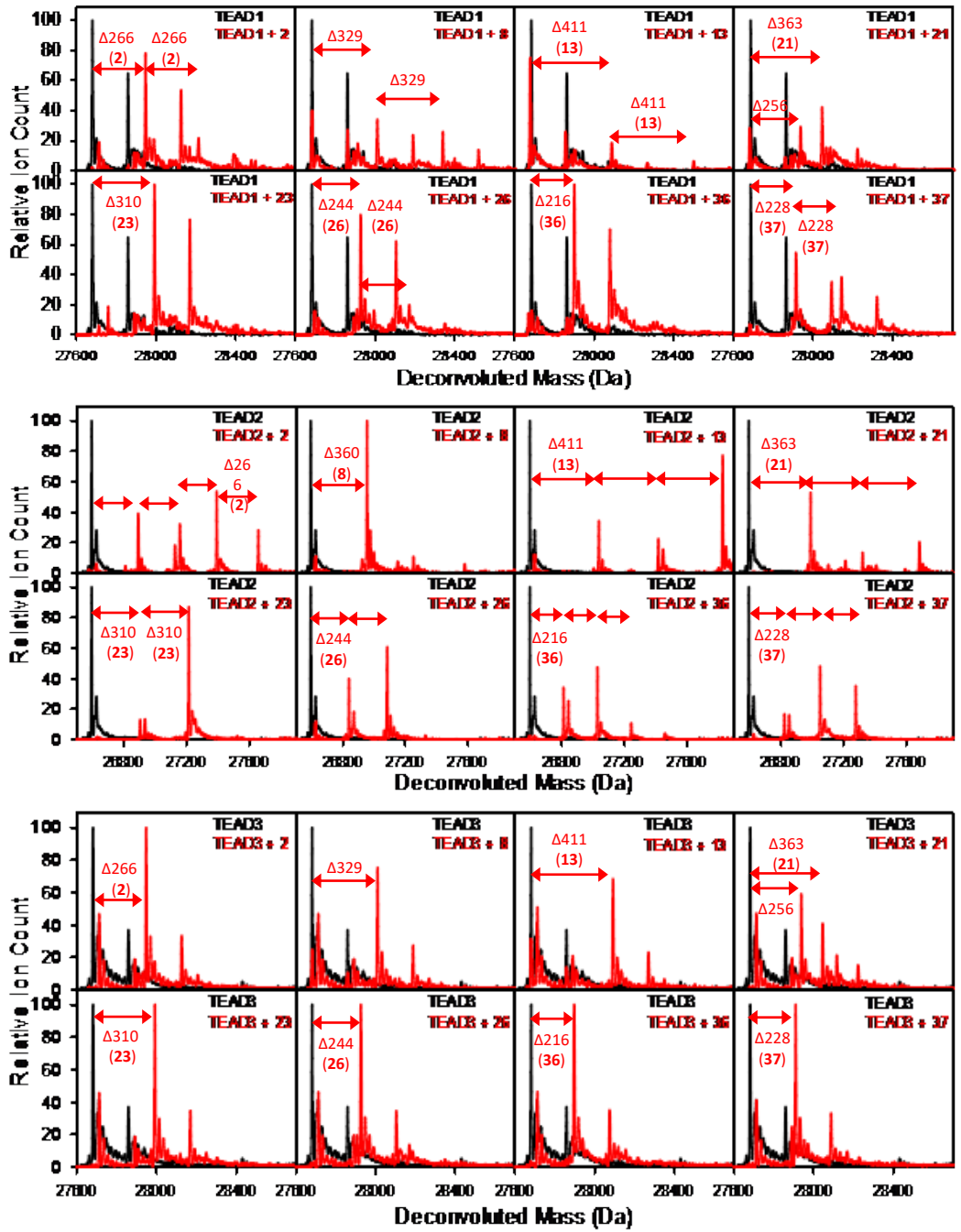


Figure S7. Reaction of compounds with TEAD1-3. Whole-protein mass spectrometry of TEAD1, TEAD2 and TEAD3 after 24 h 4°C incubation with 100 μM compound. The extent of adduct formation with

Supplementary Table S1. Whole-Protein Mass Spectrometry of TEAD1-3 Incubated with 100 µM Compound at 4°C

	Compound Adduct at 24 h 4°C (%)		
Compound	**TEAD1**	**TEAD2**	**TEAD3**
2 (RHA-059)	80, 20 [a]	0, 18, 54, 28	100
8 (RHA-363)	35 [b], 24 [b]	100	75 [b]
13 (RHA-302)	18, 7	0, 23, 77	68
21 (MAT-241)	29 [c], 42	61, 16, 23	59 [c], 41
23 (TED-564)	100	13, 87	100
26 (TED-567)	80, 20	39, 61	100
36 (RHA-337)	100	37, 51, 12	100
37 (RHA-349)	56, 38	17, 48, 35	100

[a] Additional protein-compound adduct detected in the mass spectrum

[b] Compound **8** (RHA-363) adduct to TEAD1 and TEAD3 was 329 Da instead of the expected 360 Da.

[c] Compound **21** (MAT-241) formed an additional 256 Da adduct to TEAD1 and TEAD3.