Adaptation of bound YAP to mutations at its binding interface with TEAD.

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Supplementary Tables

Parameters	wt^{YAP} wt^{TEAD4}	wt^{YAP} $Glu263Ala^{TEAD4}$	wt^{YAP} $Tyr429Phe^{TEAD4}$	
	Data set			
PDB code	6GE3	6GE4	6GE5	6GEK
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P2_12_12_1$
Cell parameters (Å; deg)	a=b=59.1, c=150.0	a=b=59.0, c=159.1	a=b=59.0, c=159.8	a=43.2, b=77.0, c=164.0
Number of complexes per ASU[a]	1	1	1	2
Resolution (Å)	1.85 (1.90-1.85)	1.97 (2.02-1.97)	2.05 (2.10-2.05)	2.28 (2.34-2.28)
Unique reflections	25036	20703	18467	25747
R_{merge}	0.068 (0.704)	0.088 (1.007)	0.12 (0.863)	0.11 (0.651)
I/σ(I)	26.3 (3.6)	22.8 (3.9)	20.0 (3.6)	12.1 (2.7)
Completeness (%)	99.8 (100.0)	99.9 (93.7)	99.8 (100.0)	99.7 (100.0)
Redundancy	12.8 (12.9)	12.7 (13.3)	12.8 (12.8)	6.3 (6.9)
	Refinement			
Resolution (Å)	1.85 (1.90-1.85)	1.97 (2.02-1.97)	2.05 (2.10-2.05)	2.28 (2.34-2.28)
Number of reflections used	23715 (1689)	19666 (1390)	17542 (1254)	24458 (1735)
Fraction of test set for calculating R_{free} (%)	5.0	5.0	5.0	5.0
Number of reflections in the test set	1249 (89)	1036 (74)	924 (66)	1288 (92)
R factor (R_{work}/R_{free})	0.218/0.242 (0.287/0.368)	0.224/0.241 (0.284/0.296)	0.223/0.238 (0.261/0.273)	0.219/0.247 (0.281/0.300)
R.m.s.d. bond lengths (Å) / bond angles (°)	0.007/1.066	0.006/0.958	0.006/0.964	0.007/0.951
Number of atoms (protein+myristate/ water+buffer/YAP)	1736/201/316	1767/147/324	1756/216/324	3437/190/526
Myristate[b]	non-covalent	covalent	covalent	covalent

Parameters	wt^{YAP} Glu263Ala-Tyr429Phe^{TEAD4}	Ser94Ala^{YAP} wt^{TEAD4}	Ser94Ala^{YAP} Glu263Ala^{TEAD4}	Ser94Ala^{YAP} Tyr429Phe^{TEAD4}
	Data set			
PDB code	6GE6	6GEC	6GEE	6GEG
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell parameters (Å; deg)	a=b=58.9, c=158.6	a=b=58.9, c=158.5	a=b=59.3, c=158.3	a=b=58.7, c=160.6
Number of complexes per ASU[a]	1	1	1	1
Resolution (Å)	1.80 (1.85-1.80)	1.70 (1.74-1.70)	1.96 (2.01-1.96)	2.23 (2.29-2.23)
Unique reflections	26774	31611	21126	14393
R_{merge}	0.085 (0.812)	0.079 (1.011)	0.116 (0.982)	0.148 (0.994)
I/σ(I)	23.0 (3.8)	21.8 (3.3)	16.4 (3.0)	15.7 (3.1)
Completeness (%)	99.9 (99.9)	99.9 (100.0)	99.8 (100.0)	99.7 (100.0)
Redundancy	12.8 (13.1)	12.8 (13.3)	12.7 (13.2)	12.7 (12.6)
	Refinement			
Resolution (Å)	1.80 (1.85-1.80)	1.70 (1.74-1.70)	1.96 (2.01-1.96)	2.23 (2.29-2.23)
Number of reflections used	25434 (1827)	30030 (2178)	20068 (1437)	13673 (970)
Fraction of test set for calculating R_{free} (%)	5.0	5.0	5.0	5.0
Number of reflections in the test set	1339 (96)	1581 (115)	1057 (76)	720 (51)
R factor (R_{work}/R_{free})	0.219/0.239 (0.273/0.271)	0.220/0.242 (0.289/0.317)	0.216/0.245 (0.250/0.263)	0.208/0.236 (0.250/0.319)
R.m.s.d. bond lengths (Å) / bond angles (°)	0.006/0.931	0.006/0.960	0.006/0.950	0.006/0.988
Number of atoms (protein+myristate/ water+buffer/YAP)	1829/217/321	1769/205/318	1769/223/333	1760/194/337
Myristate[b]	Covalent	Covalent	Covalent	Covalent

Parameters	Ser94Ala^{YAP} Glu263Ala-Tyr429Phe^{TEAD4}
	Data set
PDB code	6GEI
Space group	$P4_12_12$
Cell parameters (Å; deg)	a=b=59.0, c=159.0
Number of complexes per ASU[a]	1
Resolution (Å)	1.65 (1.69-1.65)
Unique reflections	34774
R_{merge}	0.079 (0.910)
I/σ(I)	24.7 (3.2)
Completeness (%)	99.9 (100.0)
Redundancy	12.9 (13.4)
	Refinement
Resolution (Å)	1.65 (1.69-1.65)
Number of reflections used	33036 (2397)
Fraction of test set for calculating R_{free} (%)	5.0
Number of reflections in the test set	1739 (126)
R factor (R_{work}/R_{free})	0.229/0.248 (0.325/0.356)
R.m.s.d. bond lengths (Å) / bond angles (°)	0.006/0.960
Number of atoms (protein+myristate/ water+buffer/YAP)	1846/230/337
Myristate[b]	Covalent

Supplementary Table 1. X-ray data collection and refinement statistics. Values in brackets indicate the specific values in the last resolution shell. [a]. ASU: asymmetric unit. [b]. The myristate moiety was modelled either non-covalently or covalently bound to TEAD4 via Cys367 or Lys344 (in many cases the electron density indicated a possible mixture of the bound forms, but

only one form was modelled according to the most likely major species). In all the structures, the fatty acid occupies the same binding pocket.

YAP:TEAD complexes	Asp93^{YAP}		Ser/Ala94^{YAP}		Phe95^{YAP}	
	Φ (°)	**Ψ (°)**	**Φ (°)**	**Ψ (°)**	**Φ (°)**	**Ψ (°)**
wt^{YAP}:wt^{TEAD4}	-57.64	-33.87	-62.77	-20.35	-58.00	-28.34
wt^{YAP}:Glu263Ala^{TEAD4}	-59.45	-32.13	-60.94	-21.54	-61.27	-29.39
wt^{YAP}:Tyr429Phe^{TEAD4}	-54.75	-39.48	-64.83	-17.26	-61.83	-31.99
wt^{YAP}:Glu263Ala-Tyr429Phe^{TEAD4}	-58.33	-32.15	-68.40	-14.21	-68.43	-27.43
Ser94Ala^{YAP}:wt^{TEAD4}	-55.86	-42.63	-97.76	19.84	-59.72	-30.25
Ser94Ala^{YAP}:Glu263Ala^{TEAD4}	-52.46	-47.05	-96.44	20.99	-59.78	-30.06
Ser94Ala^{YAP}:Tyr429Phe^{TEAD4}	-59.51	-38.25	-58.70	-18.98	-67.10	-31.81
Ser94Ala^{YAP}:Glu263Ala-Tyr429Phe^{TEAD4}	-56.52	-36.37	-63.81	-17.27	-66.30	-32.40

Supplementary Table 2. Φ and Ψ dihedral angles of the YAP residues 93, 94 and 95 in the different YAP:TEAD complexes.

Legend to Supplementary Figures

Supplementary Fig. S1. Structures of the wt^{YAP}:$Tyr429Phe^{TEAD4}$ complex solved from different crystal forms. The structures of the wt^{YAP}:wt^{TEAD4} (magenta), wt^{YAP}:$Tyr429Phe^{TEAD4}$ space group $P4_12_12$ (cyan) and $P2_12_12_1$ (green) have been superimposed. The position of Tyr/$Phe429^{TEAD4}$ and $Ser94^{YAP}$ is indicated. The red arrow shows the observed movement of YAP.

Supplementary Fig. S2. Effect of proton exchange with buffer on ΔH_{obs}. To determine the influence of protonation/deprotonation effects on the measurement of the binding enthalpy (ΔH_{obs}) of four different YAP:TEAD complexes, plots of ΔH_{obs} versus ΔH_{ioni} (ionization enthalpy of the buffer) were constructed. These plots were analysed by linear regression (GraphPad Prism, GraphPad Software, Inc., La Jolla, CA) using the equation: $\Delta H_{obs} = \Delta H_{nbe} + n_{H+}.\ \Delta H_{ioni}$, where ΔH_{nbe} is the enthalpy measured in the absence of buffer effect and n_{H+} the number of protons exchanged with buffer during binding. ΔH_{ioni} for PIPES, HEPES and TRIS were taken as 2.68, 4.88 and 11.34 kcal/mol, respectively [1].

Supplementary Fig. S3. LC-MS analyses of the YAP and TEAD4 proteins. The panels are representative HPLC profiles of the proteins used in this study. The numbers represent the mass of the proteins as determined by mass spectrometry. The numbers in brackets correspond to the theoretical mass of the proteins calculated from their primary sequence. For the TEAD4 proteins, the underlined numbers correspond to myristoylated TEAD4 (major form) and the other numbers to palmitoylated TEAD4 (minor form) (see Mesrouze et al., 2017 for more details [2]). The amount of protein used for the HPLC analysis is indicated.

Supplementary Fig. S4. Surface Plasmon Resonance. The N-biotinylated-Avitagged TEAD4 proteins were immobilized on sensor chips and the binding of different concentrations of wt/$Ser94Ala^{YAP}$ was measured at 298°K. The upper and lower panels show representative

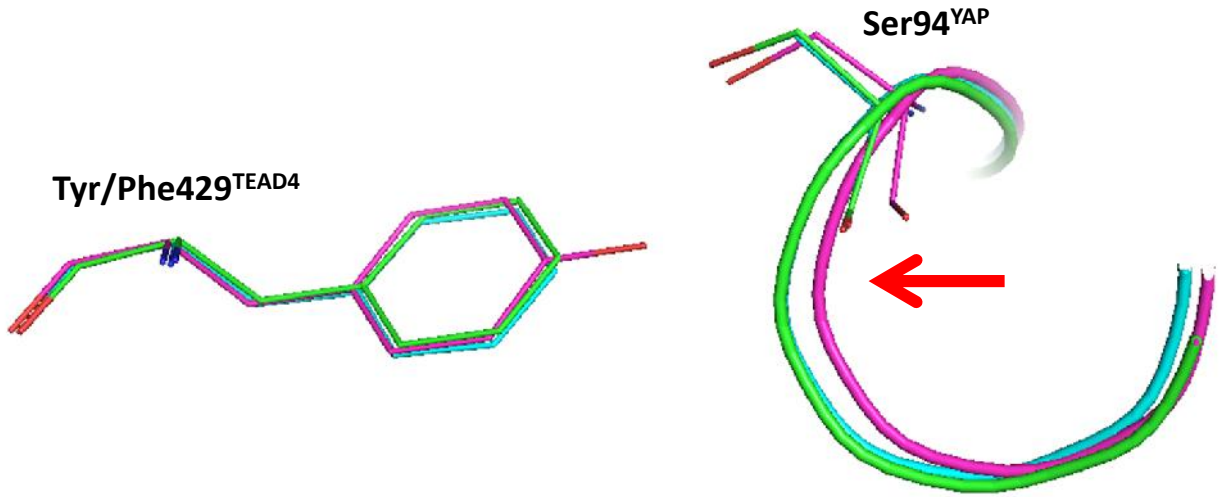
sensorgrams and the corresponding binding isotherms from which K_d values (at equilibrium) were derived.

Supplementary Fig. S5. Isothermal Titration Calorimetry. The figure shows representative thermograms obtained with the different YAP:TEAD complexes. The corresponding binding isotherms could not be represented because of a software malfunction (MicroCal PEAQ-ITC Analysing Software, Malvern Instruments, UK) that made it impossible to display reverse titration experiments properly. The manufacturers (Malvern Instruments, UK) were not able to correct this malfunction for the preparation of this manuscript.

References

1. Goldberg RN, Kishore N, Lennen RM (2002) Thermodynamic quantities for the ionization reactions of buffers. J Phys Chem Ref Data 31:231-370.
2. Mesrouze Y, Meyerhofer M, Bokhovchuk F, Fontana P, Zimmermann C, Martin T, Delaunay C, Izaac A, Kallen J, Schmelzle T, Erdmann D, Chène P (2017) Effect of the acylation of TEAD4 on its interaction with co-activators YAP and TAZ. Prot Sci 26:2399-2409.

Supplementary Fig. S1



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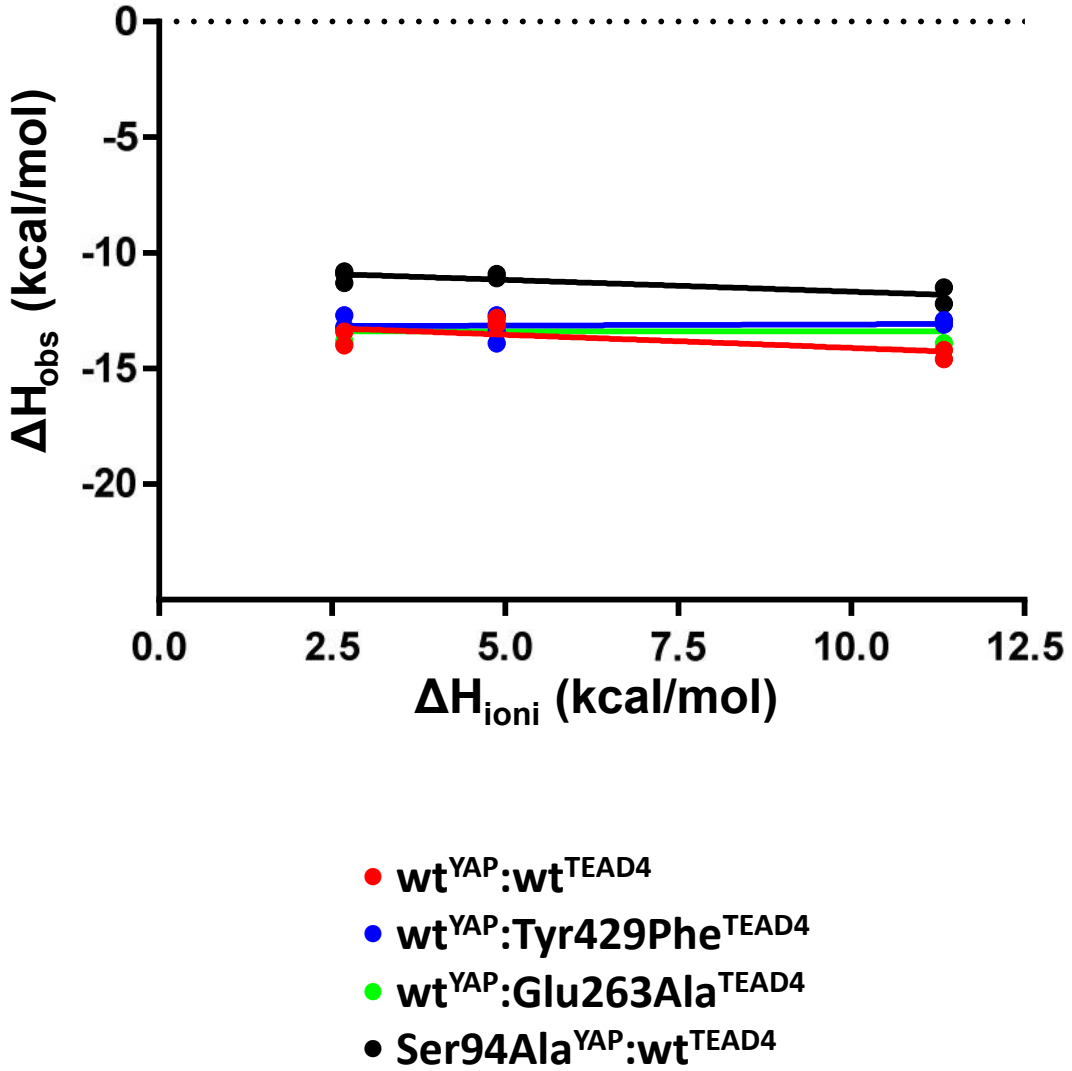

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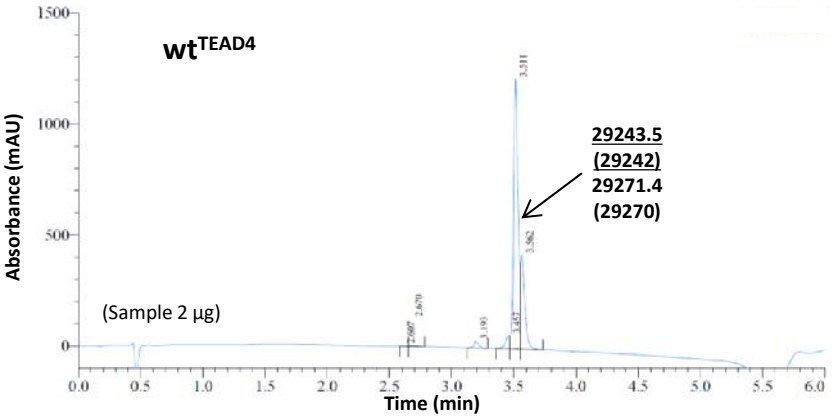
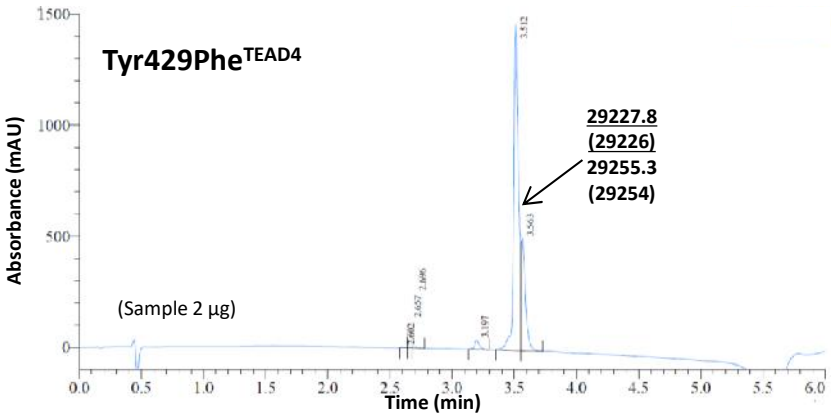
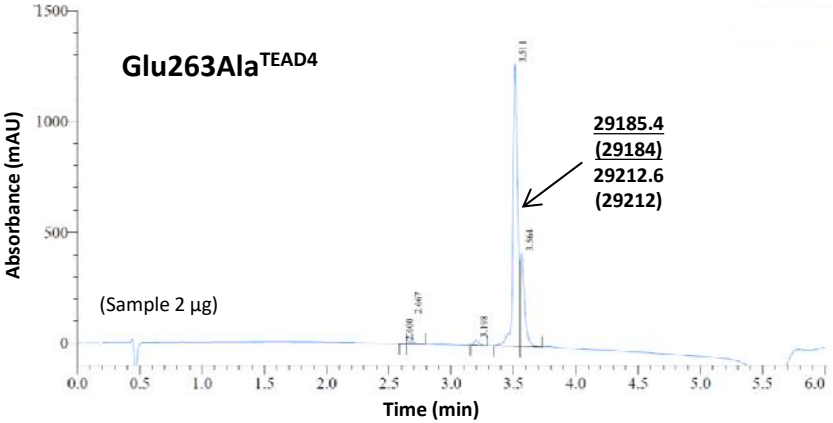
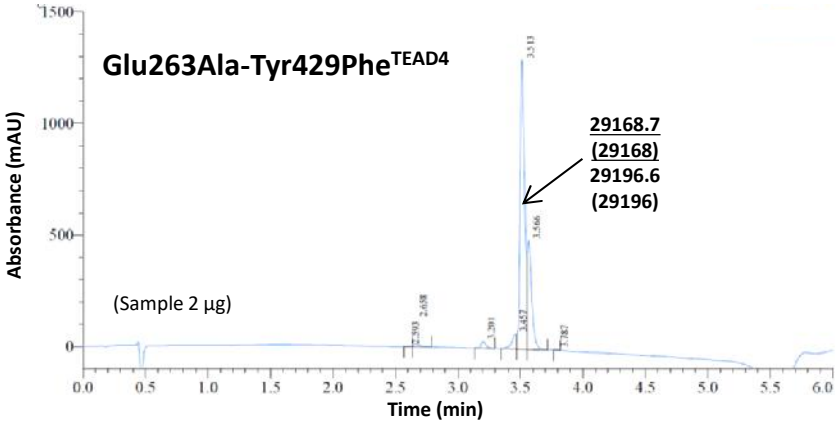
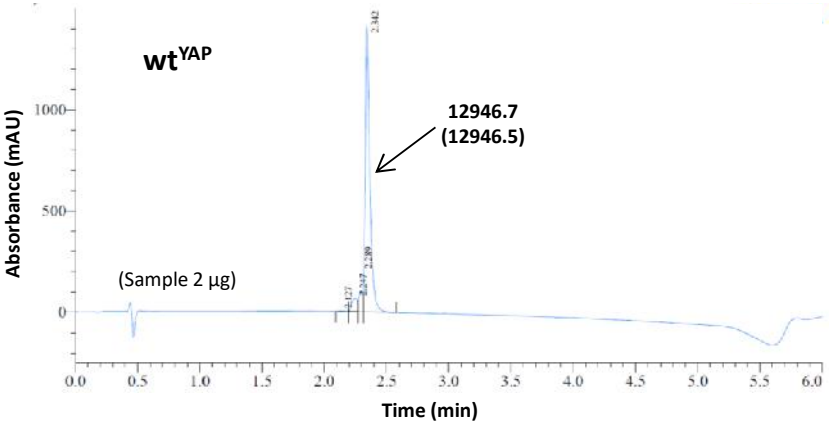
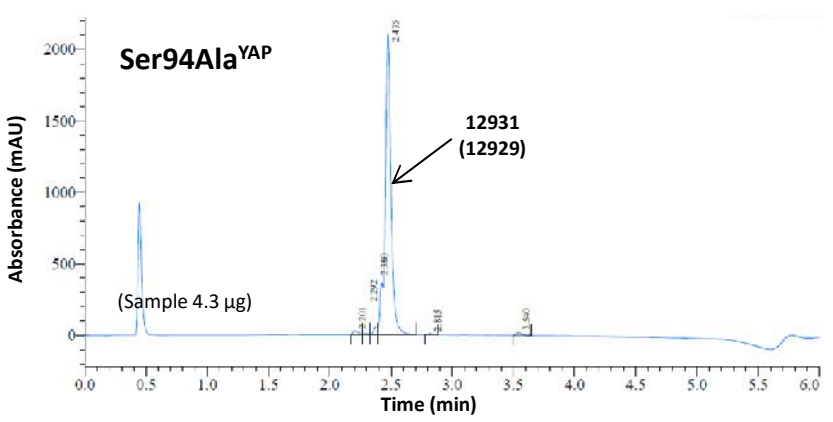




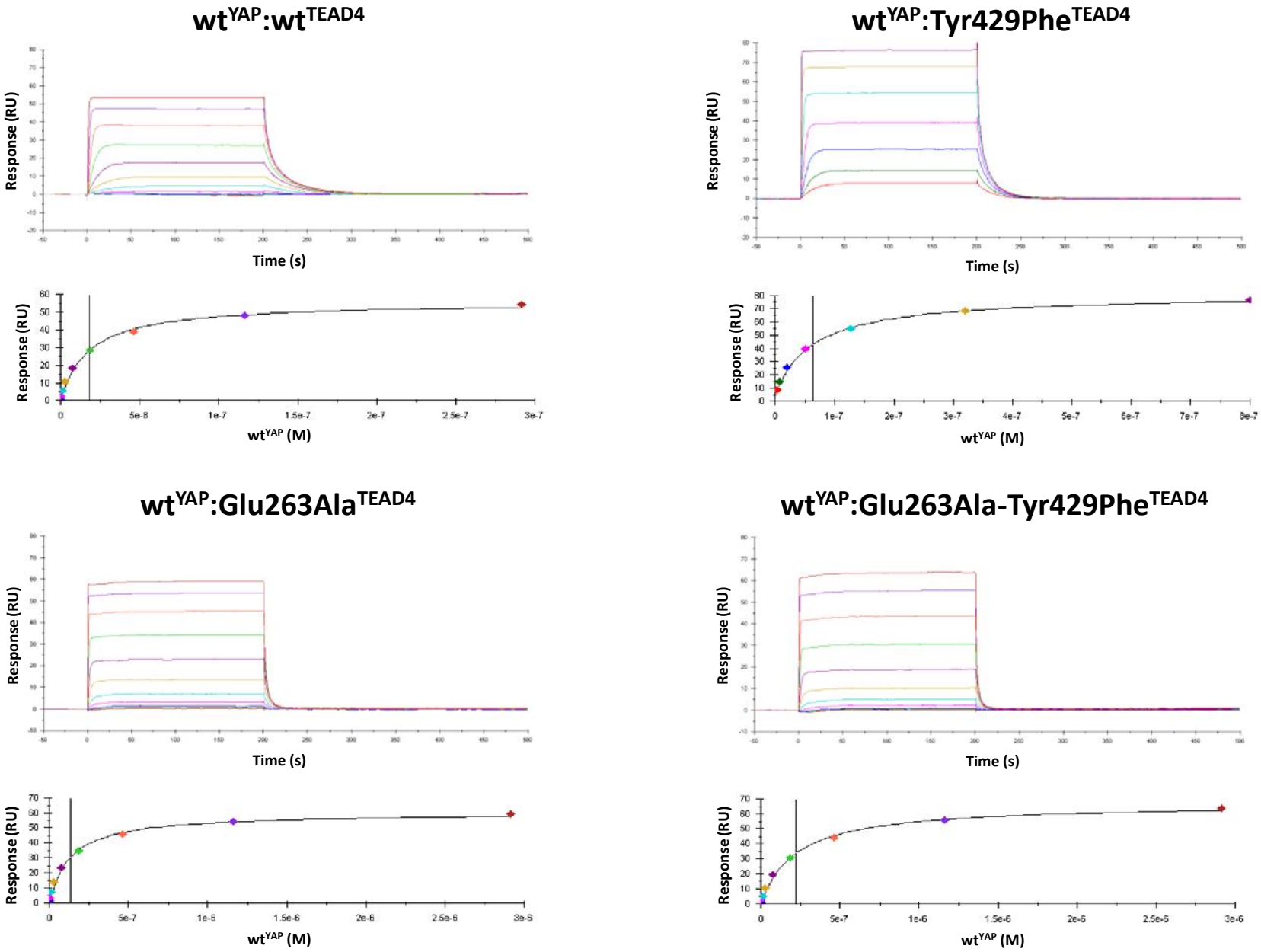








Supplementary Fig. S4



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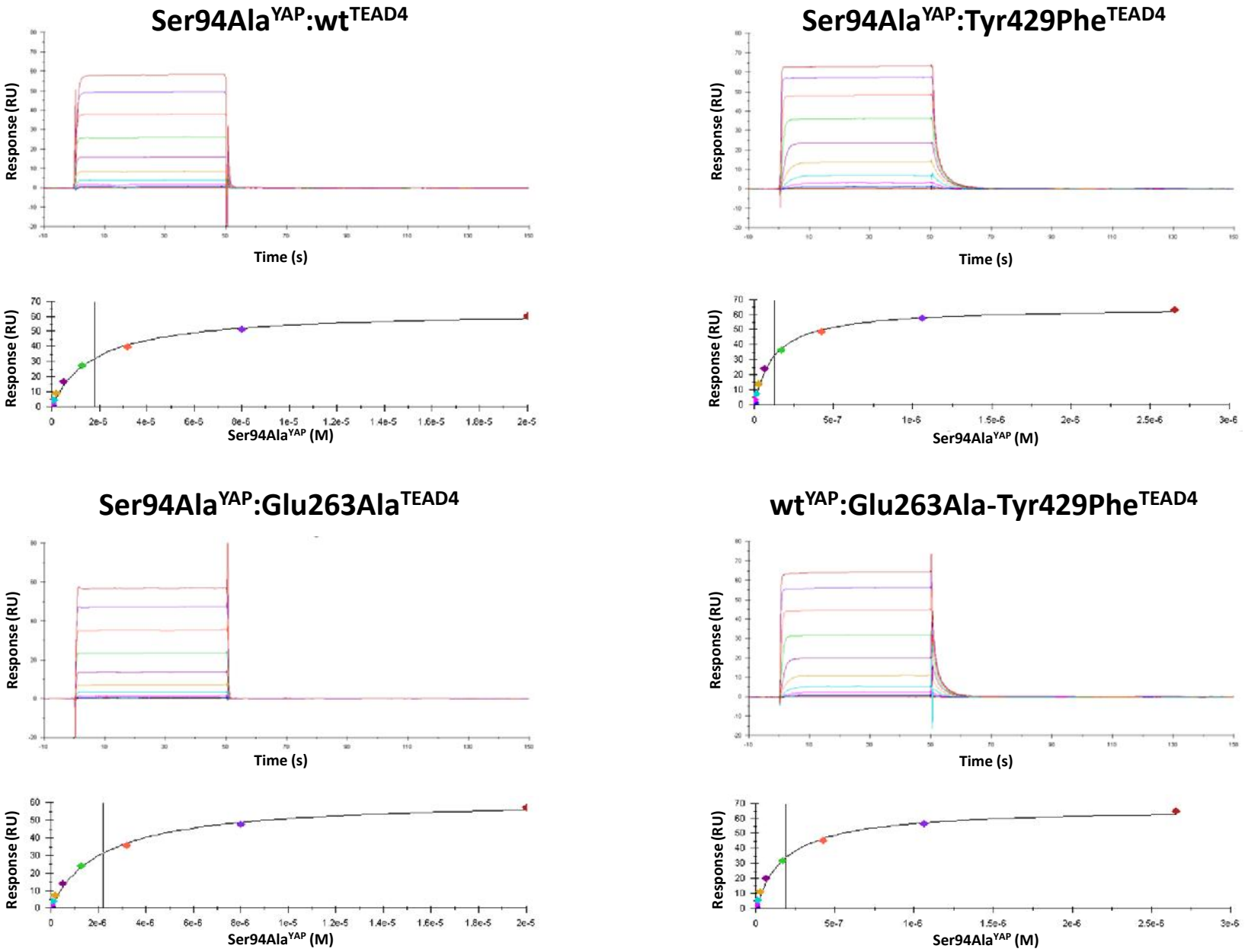

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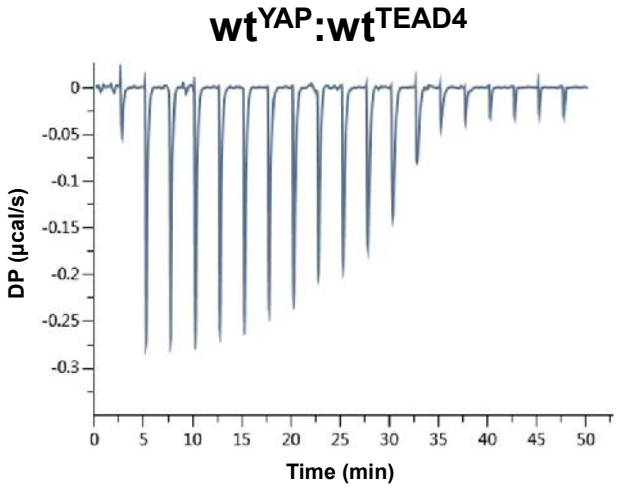

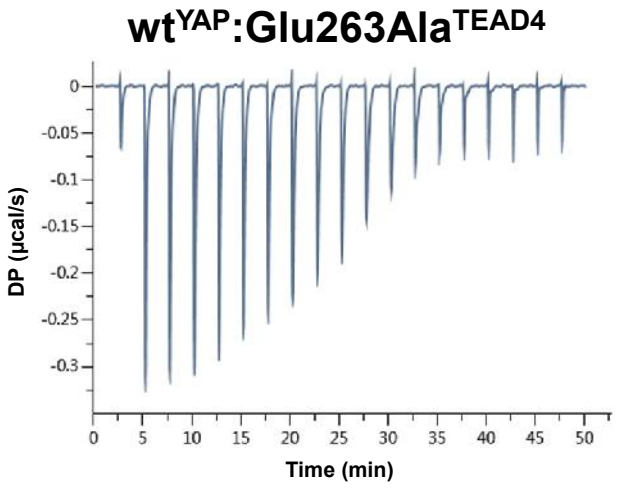

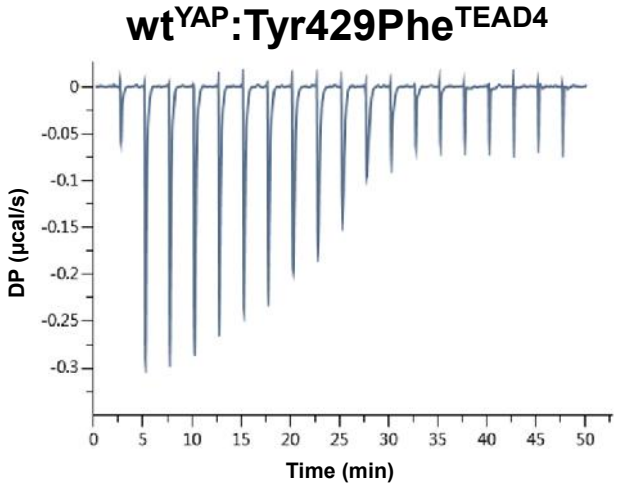

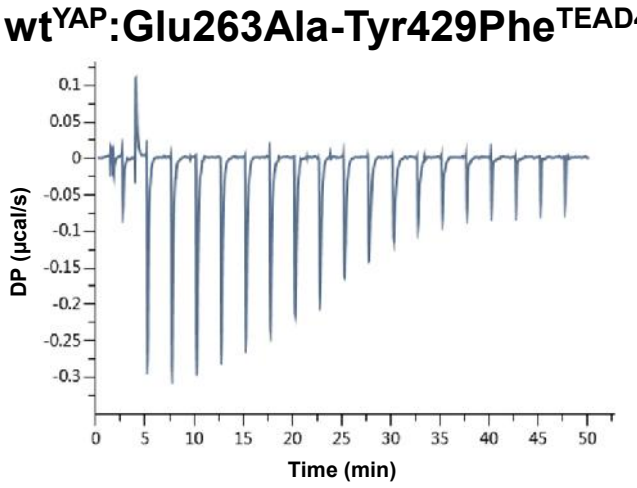

Supplementary Fig. S5