Identification of Two Secondary Ligand Binding Sites in 14-3-3 Proteins Using Fragment Screening

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Supporting Information

Table S1: Crystallographic data

	14-3-3σΔc/ TAZpS89	**14-3-3σΔc/ TAZpS89/NV1**	**14-3-3σΔc/ TAZpS89/NV2**	**14-3-3σΔc/ TAZpS89/NV3**
PDB ID	5N75	5N5R	5N5T	5N5W
Data collection				
Wavelength (Å)	1.54	1.54	1.54	1.033
Resolution (Å)	45.60-1.80 (1.84-1.80)	45.48-1.80 (1.84-1.80)	65.96-1.80 (1.84-1.80)	65.85-1.04 (1.39-1.37)
Space group	C2221	C2221	C2221	C2221
Unit cell	82.18 112.27 62.82	81.96 111.97 62.63	81.51 112.27 62.59	81.29 112.29 62.51
Total reflections[a]	167641 (6454)	168053 (6575)	167853 (6842)	788177 (38101)
Unique reflections[a]	27105 (1500)	26488 (1393)	26972 (1563)	60317 (2923)
Redundancy[a]	6.2 (4.3)	6.3 (4.7)	6.2 (4.4)	13.1 (13.0)
Completeness (%)[a]	99.6 (94.5)	98.1 (87.1)	99.8 (98.2)	100.0 (100.0)
Average $I/\sigma_{(I)}$[a]	33.3 (9.7)	38.9 (8.8)	42.1 (11.0)	20.7 (2.1)
Wilson B-factor	8.7	6.7	8.3	13.6
$CC_{1/2}$[a,b]	0.999 (0.989)	1.0 (0.98)	1.0 (0.987)	1.0 (0.756)
R_{sym}[a,c]	0.039 (0.120)	0.040 (0.169)	0.034 (0.129)	0.072 (1.348)
R_{meas}[a,d]	0.043 (0.136)	0.043 (0.190)	0.037 (0.146)	0.075 (1.403)
Refinement				
Reflections (refinement)	27083	26475	26951	60289
Reflections (R-free)	1429	1308	1325	3044
Non-hydrogen atoms (protein / solvent)	2055 / 257	2025 / 274	2029 / 248	2001 / 214
R_{work} (%)	15.3	14.9	15.9	14.4
R_{free} (%)	18.6	18.2	18.4	16.5
RMS (bonds) / (angles)	0.005 / 0.707	0.012 / 0.916	0.004 / 0.587	0.006 / 0.859
Average protein B-factor	11.93	11.68	12.35	14.22
Ramachandran: favored / outliers (%)	98.3 / 0.0	97.4 / 0.45	98.7 / 0.0	98.3 / 0.0
Clashscore	0.73	0.99	1.46	1.25

[a] Number in parentheses is for the highest resolution shell used in the refinement

[b] $CC_{1/2}$ = Pearson's intra-dataset correlation coefficient, as described by Karplus and Diederichs

[c] $R_{sym} = \sum_h \sum_l | I_{hl} - \langle I_h \rangle | / \sum_h \sum_l \langle I_h \rangle$, where I_{hl} is the intensity of the l^{th} observation of reflection h and $\langle I_h \rangle$ is the average intensity of reflection h

[d] $R_{meas} = \sum_h | \sqrt{(n_h/(n_h-1))} \sum_l | I_{hl} - \langle I_h \rangle | | / \sum_h \sum_l \langle I_h \rangle$, where n_h is the number of observations of reflection h

[e] Correlation of experimental intensities with intensities calculated from refined model, as described by Karplus and Diederichs

Karplus, P. A. & Diederichs, K. (2012). Linking crystallographic model and data quality. *Science* **336**, 1030-3.

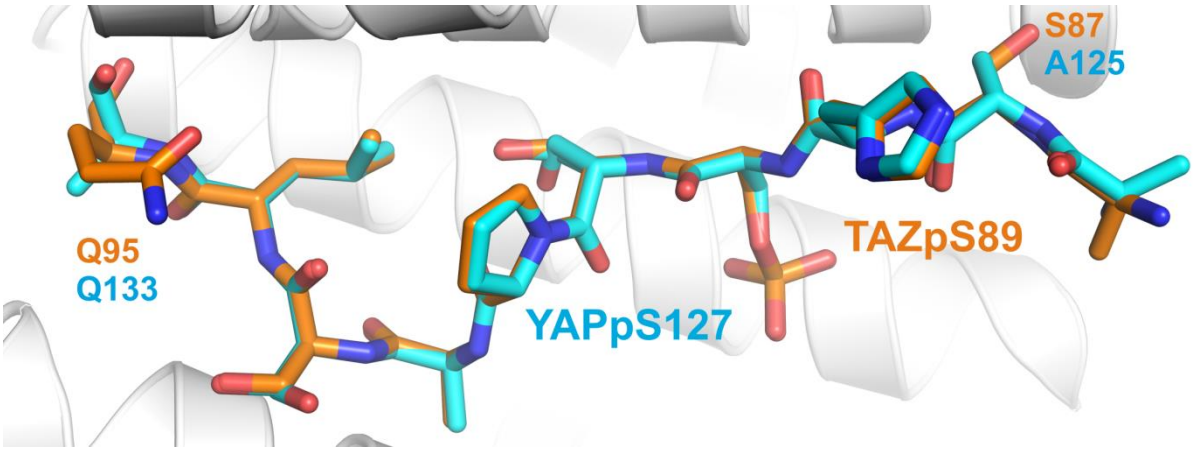

Figure S1. Overlay of YAP and TAZ peptide in complex with 14-3-3. Crystal structure of 14-3-3σ (grey, cartoon) bound to YAPpS127 (cyan sticks) and TAZpS89 (orange sticks). The only sequence difference is located at the -2 position relative to the phosphoserine (YAP: A125, TAZ: S87). (PDB IDs: 3MHR, 5N75)

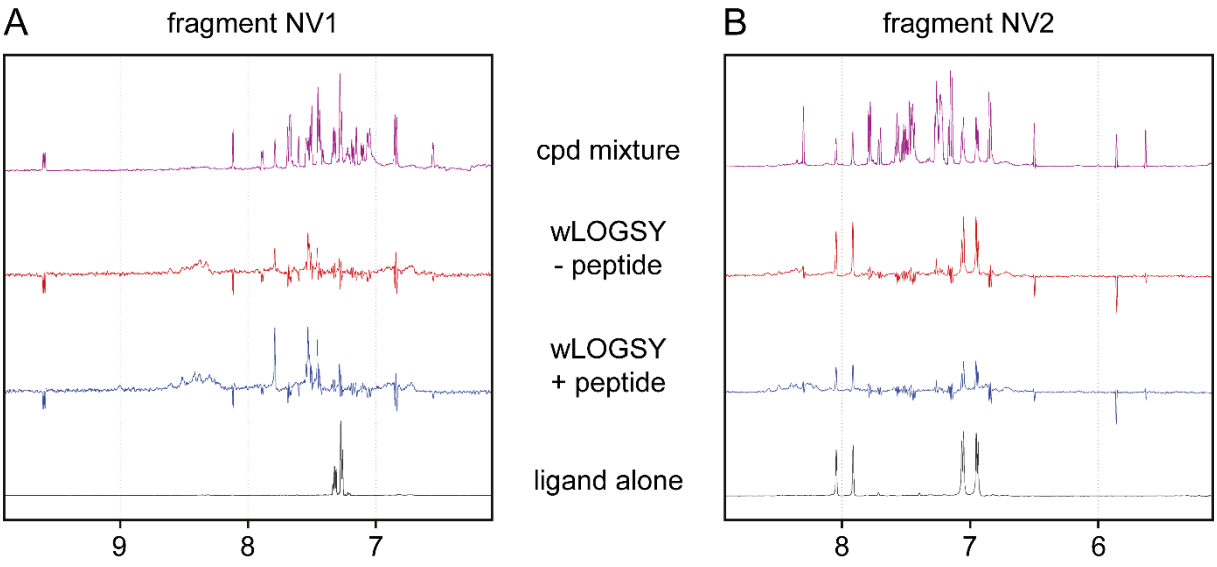

Figure S2. Binding of fragments NV1 (A) and NV2 (B) to 14-3-3 in presence and absence of TAZpS89 peptide. 1D ^{1}H spectra of compound mixtures are shown in magenta. Binding of fragments to 14-3-3 is evidenced by wLOGSY spectra recorded in absence (red) and presence (blue) of TAZpS89 peptide. 1D ^{1}H spectra of identified ligands are shown in black.

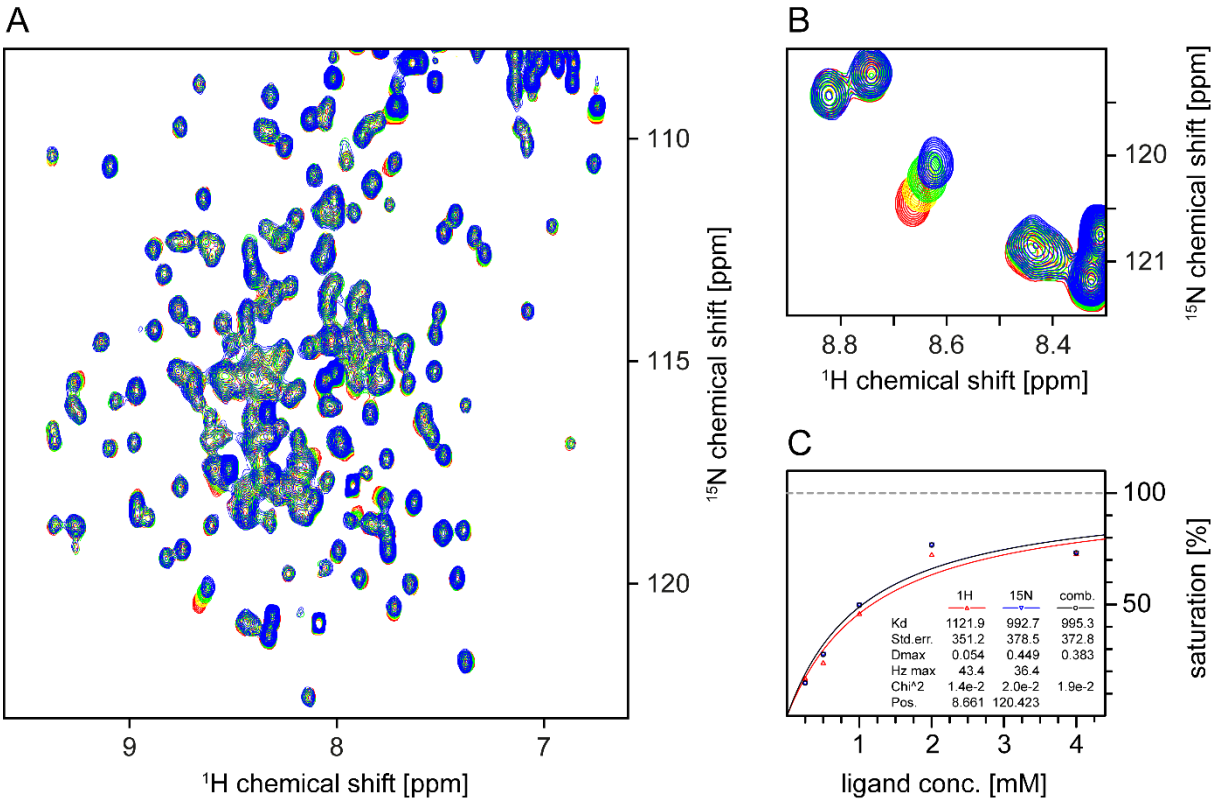

Figure S3. Determination of binding affinity of fragment NV2 to the 14-3-3 / TAZpS89 complex. (A) fragment NV2 was titrated to a preformed complex of 100 mM 14-3-3 and 150 mM TAZpS89 (red) in 5 steps: 250 µM (not shown), 500 µM (yellow), 1 mM (green), 2 mM (blue), 4 mM (not shown). (B) Excerpt of spectrum A showing the resonance with largest chemical shift perturbations. (C) Fitting of data indicates a ~1 mM affinity.

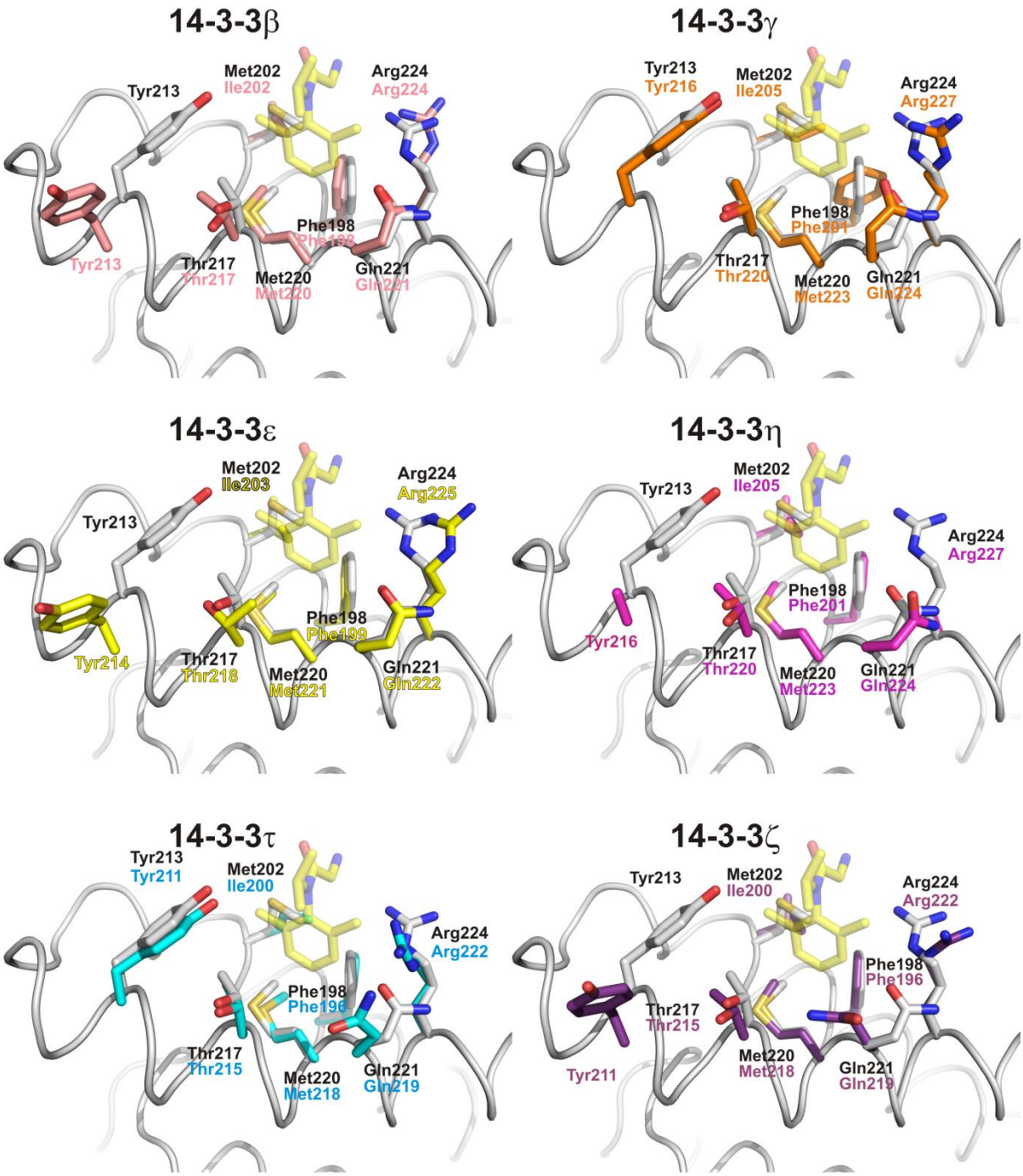

Figure S4. **Structural alignments of the NV1 binding pocket among the seven human 14-3-3 isoforms**. Structures with the following PDB IDs were used for the depicted alignments: 14-3-3β (4GNT), 14-3-3γ (4J6S), 14-3-3ε (3UBW), 14-3-3η (2C74), 14-3-3τ (2BTP), 14-3-3ζ (4WRQ).

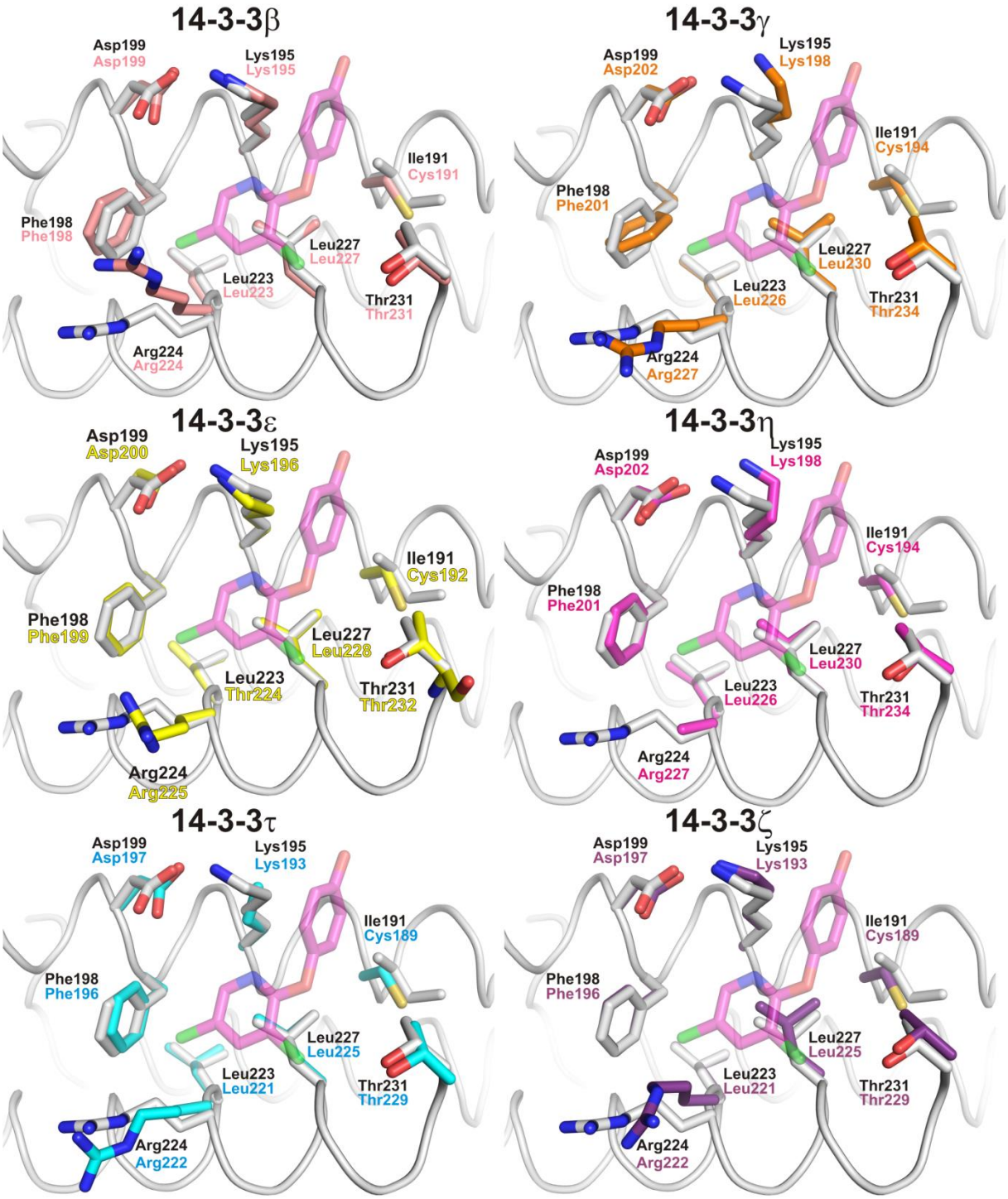

Figure S5: **Structural alignments of the NV2 and NV3 binding pocket among the seven human 14-3-3 isoforms.** Structures with the following PDB IDs were used for the depicted alignments: 14-3-3β (4GNT), 14-3-3γ (4J6S), 14-3-3ε (3UBW), 14-3-3η (2C74), 14-3-3τ (2BTP), 14-3-3ζ (4WRQ).