Interaction of an IκBα peptide with 14-3-3

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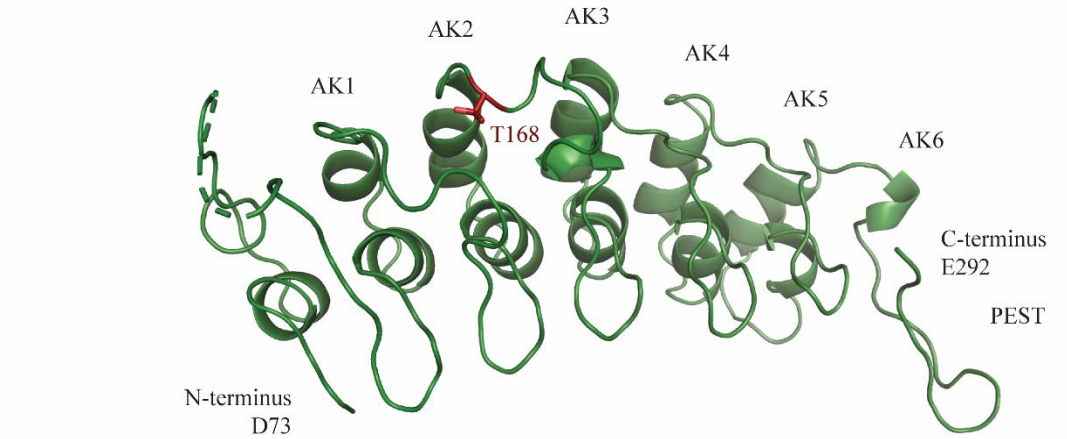
Supporting Information

SI Table 1: Results of the 14-3-3pred webserver for IκBα (uniprot ID: P25963). Shown are the phosphorylated residue (site), the sequence of IκBα from position -6 to +4 of the WT sequence around the phosphorylated residue (peptide), the scores for three different methods to estimate the likeliness of an 14-3-3 binding motif (ANN, PSSM, SVM) and the overall score (consensus) based on a combination of the three scores named before. Finally, an indication is given if information about the phosphorylation status of the predicted phospho-site are available. These results were downloaded on the 30.01.2019.

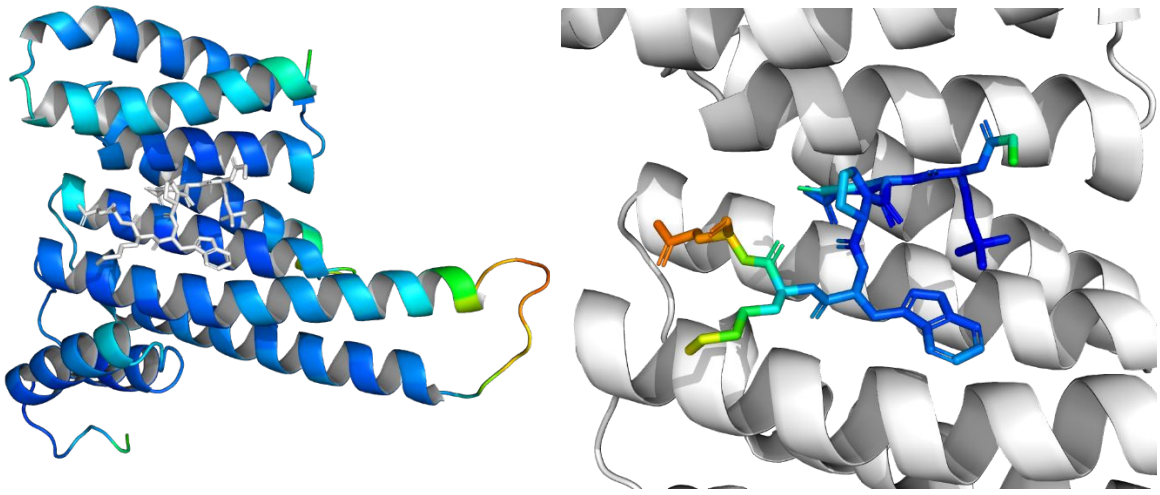
Site	Peptide_[-6:4]	ANN	PSSM	SVM	Consensus	pSer/Thr
32	LDDRHDsGLDS	0.35	0.63	-0.324	0.219	Yes
36	HDSGLDsMKDE	0.078	-0.128	-1.268	-0.44	Yes
63	QEVPRGsEPWK	0.499	0.48	-0.112	0.289	-
71	PWKQQLtEDGD	0.325	0.11	-0.251	0.061	-
76	LTEDGDsFLHL	0.097	-0.198	-1.178	-0.426	-
90	HEEKALtMEVI	0.342	0.256	-0.384	0.071	-
113	QNNLQQtPLHL	0.023	-0.5	-1.848	-0.775	-
121	LHLAVItNQPE	0.134	-0.225	-1.259	-0.45	-
146	RDFRGNtPLHL	0.18	0.451	-0.591	0.013	-
159	EQGCLAsVGVL	0.17	0.027	-0.81	-0.204	-
164	ASVGVLtQSCT	0.046	-0.316	-1.79	-0.687	-
166	VGVLTQsCTTP	0.1	-0.079	-1.136	-0.372	-
168	VLTQSCtTPHL	0.842	0.742	0.837	0.807	-
169	LTQSCTtPHLH	0.108	-0.414	-1.365	-0.557	-
174	TTPHLHsILKA	0.063	-0.217	-1.46	-0.538	-
179	HSILKAtNYNG	0.133	-0.02	-1.256	-0.381	-
185	TNYNGHtCLHL	0.054	-0.073	-1.092	-0.37	-
191	TCLHLAsIHGY	0.243	-0.085	-0.872	-0.238	-
204	IVELLVsLGAD	0.645	0.155	0.206	0.335	-
219	EPCNGRtALHL	0.033	-0.185	-1.581	-0.578	-
234	QNPDLVsLLLK	0.153	-0.196	-1.015	-0.353	-
247	ADVNRVtYQGY	0.288	-0.055	-0.575	-0.114	-
252	VTYQGYsPYQL	0.066	-0.348	-1.086	-0.456	-
257	YSPYQLtWGRP	0.063	-0.183	-1.387	-0.502	-
262	LTWGRPsTRIQ	0.218	0.004	-0.784	-0.187	-
263	TWGRPStRIQQ	0.195	0.511	-0.389	0.106	-
273	QQLGQLtLENL	0.1	0.033	-0.828	-0.232	-
283	LQMLPEsEDEE	0.15	-0.056	-0.779	-0.228	Yes
288	ESEDEEsYDTE	0.104	-0.284	-1.373	-0.517	Yes

291	DEESYDtESEF	0.15	-0.206	-1.323	-0.46	Yes
293	ESYDTEsEFTE	0.089	-0.104	-1.18	-0.398	Yes
296	DTESEFtEFTE	0.266	-0.119	-0.93	-0.261	-
299	SEFTEFtEDEL	0.235	0.057	-0.944	-0.217	Yes
316	FGGQRLtL---	0.421	0.366	-0.091	0.232	-



SI Figure 1: Crystal structure of IκBα (green cartoon; PDB ID: 1IKN). p65 and p50 are not shown for clarity. 219 out of 317 residues were visible in the electron density (D73 – E292) and structural features are indicated (AR: ankyrin repeat; PEST: proline, glutamate, serine and threonine rich sequence). Highlighted is the only predicted 14-3-3 binding site T168. (red sticks).

A **B**



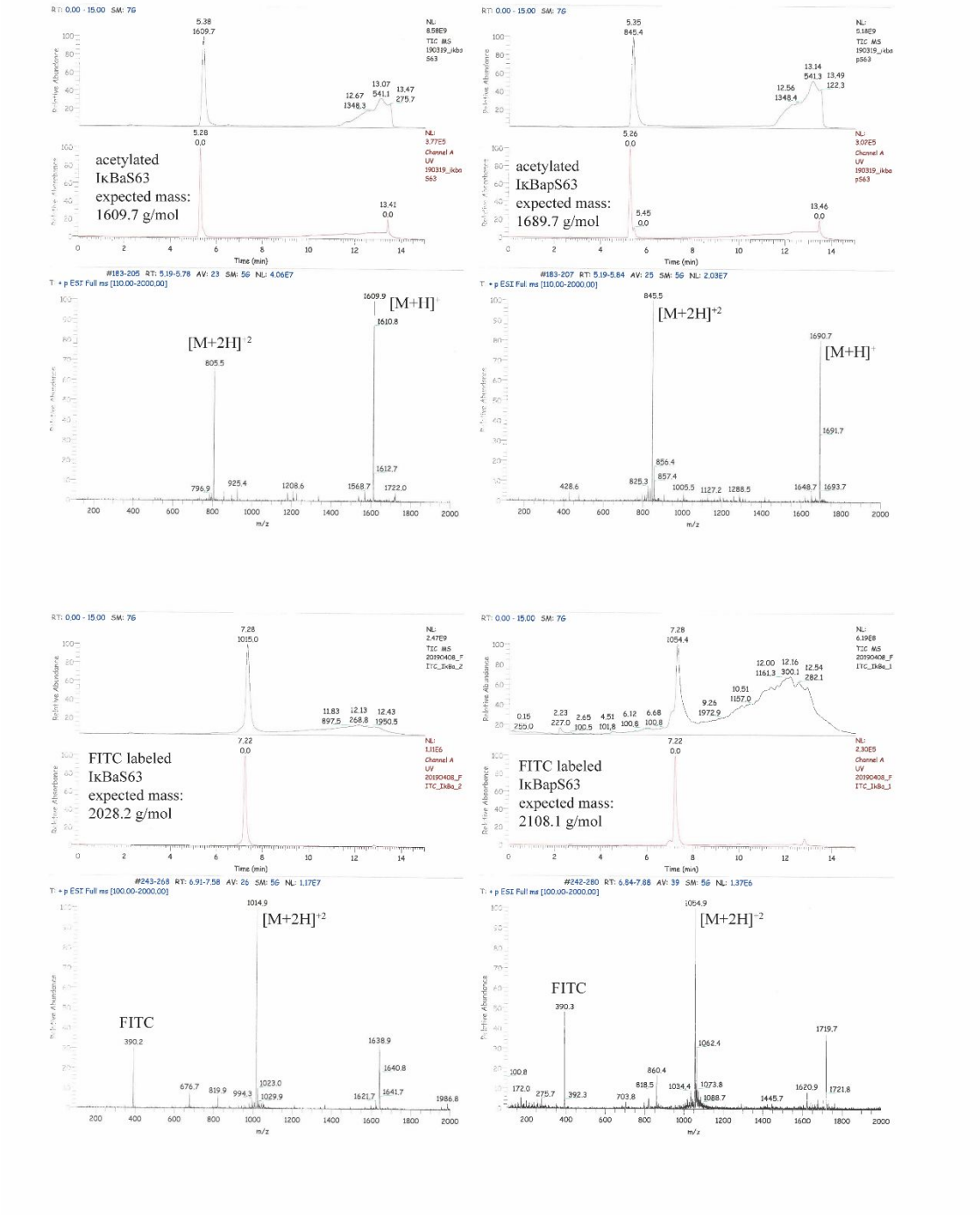
SI Figure 2: B-factor coloring of 14-3-3σΔC and the IκBαpS63 peptide. A The 14-3-3σΔC protein is represented as cartoon while the IκBαpS63 peptide is shown as white sticks. **B** The IκBαpS63 peptide is shown as sticks while the 14-3-3σΔC protein is shown as white cartoon. The B-factor coloring follows the rainbow color scheme with blue for low B-factors and red for high B-factors. The B-factors vary between 4.6 – 57.04 A^2.

SI Table 2: Crystallographic statistics of the X-ray structure of the IκBαpS63/14-3-3σΔC232-248 complex (PDB ID: 6Y1J). Values in parentheses show statistics for the high resolution shell.

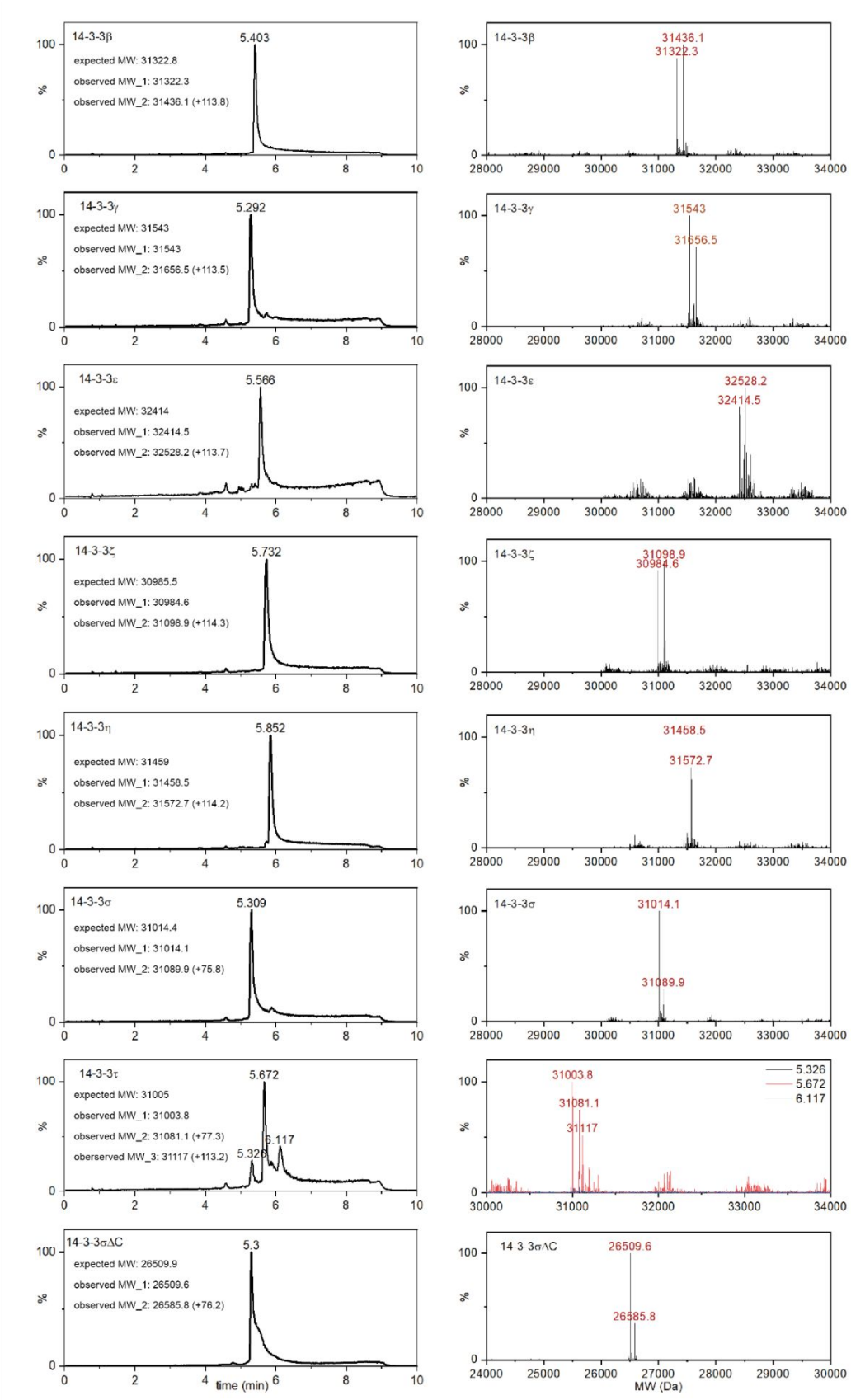
Property	Value	Source
Space group	C 2 2 21	phenix_table1
Cell constants a, b, c, α, β, γ	82.46Å 111.82Å 62.48Å 90.00° 90.00° 90.00°	phenix_table1
Resolution (Å)	45.49 - 1.13 (1.17-1.13) 45.49 - 1.13	phenix_table1 EDS
% Data completeness (in resolution range)	100.0 (99.97) 100.0	phenix_table1 EDS
Unique reflections	107826 (10678)	phenix_table1
Multiplicity	2.0 (2.0)	phenix_table1
< I/σ(I) >	15.53 (2.43)	Xtriage
CC1/2	1 (0.903)	phenix_table1
Wilson B-factor (A^2)	9.6	Xtriage
Rmerge	0.0217 (0.221)	phenix_table1
Rmeas	0.0307 (0.313)	phenix_table1
Rpim	0.0217 (0.221)	phenix_table1
Anisotropy	0.434	Xtriage
Bulk solvent ksol(e/ Å 3), Bsol(Å2)	0.40 , 44.5	EDS
L-test for twinning	< |L| > = 0.49, < L2 > = 0.33	Xtriage
Estimated twinning fraction	No twinning to report	Xtriage
Fo,Fc correlation	0.97	EDS
Refinement program	PHENIX (1.11.1_2,57,5)	Depositor
R, Rfree	0.170 (0.271), 0.183 (0.279) 0.171 , 0.182	phenix_table1 DCC
Rfree test set	5463 reflections (5.03%)	wwPDI3-VP
Total number of atoms	4545	wwPDI3-VP
Ramachandran favored (%)	97.86	phenix_table1
Ramachandran allowed (%)	2.14	phenix_table1
Ramachandran outliers (%)	0.00	phenix_table1
Rotamer outliers (%)	0.00	phenix_table1
RMS bonds, angles	0.008, 1.28	phenix_table1
Clashscore	2.83	phenix_table1
Average B, all atoms (Å2)	14.0 14.2	wwPDI3-VP phenix_table1
Average B, macromolecules	11.6	phenix_table1
Average B, ligands	50.1	phenix_table1
Average B, solvent	25.6	phenix_table1

SI Table 3: Quantitative comparison of 14-3-3 binding motifs. The analyzed structures are grouped based on the subpanels of Figure 4 and the peptide sequences were aligned via the phosphorylated residue of the binding motifs (position 0). The complete structures (14-3-3 protein and interaction partner) were aligned via the Cα atoms of the residues and the distances of corresponding amino acids were measured using the "Structural alignment – Gesamt" tool of the CCP4i2 suite. Due to the considerably different conformations of the peptides, the software aligned position +3 of 6F09 with position +4 of the reference structure (6HEO- IκBα) and position +4 of 5N6N0 with the position +5 of 6HEO (new positions are indicated in parentheses).

PDB ID\ position	-3	-2	-1	**0**	1	2	3	4	5
Sequence									
reference model 6Y1J			GLY	**SEP**	GLU	PRO	TRP	LYS	GLN
Figure 4A 6QZR	ARG	SER	CYS	**TPO**	PRO	LEU	PRO		
Figure 4B 6F09			TYR	**SEP**	SER	PRO	-	ASP (+3)	ILE (+4)
1O9F	GLN	SER	TYR	**TPO**	VAL				
3E6Y	GLN	SER	TYR	**TPO**	VAL				
Figure 4C 5ULO		SER	PRO	**SEP**	PHE				
4DAU		ALA	MET	**SEP**	PHE	GLN	SER		
Figure 4D 5N6N	ARG	ARG	GLY	**SEP**	GLU	ASP	ASP	-	THR (+4)
Figure 4E 6GKF	TYR	ASP	LEU	**SEP**	LEU	PRO	PHE	PRO	
3UAL	ARG	SER	PHE	**SEP**	GLU	PRO	PHE	GLY	
Distance (Å)									
Figure 4A 6QZR			0.51	**0.53**	0.71	2.41	3.07		
Figure 4B 6F09			0.50	**0.25**	0.26	0.79	-	2.81	1.58
1O9F			0.67	**0.71**	1.44				
3E6Y			0.60	**0.72**	1.14				
Figure 4C 5ULO			0.91	**0.56**	0.63				
4DAU			0.52	**0.38**	0.37	0.42			
Figure 4D 5N6N			1.48	**0.80**	0.81	3.09	2.64	-	1.58
Figure 4E 6GKF			1.36	**0.50**	0.81	1.61	2.63		
3UAL			0.36	**0.22**	0.33	0.96	1.53	2.96	



SI Figure 3: LC-MS analysis of the in-house synthesized and purified peptides.



SI Figure 4: QTOF measurements of the purified 14-3-3 isoforms. The purity of the chromatogram (left panel) was taken as indication for the purity of the protein. The mass over

charge distributions corresponding to the peak(s) in the chromatogram were deconvoluted to a single mass using the the MaxEnt1 function of the MassLynx MS Software. Settings were chosen in the following way: the output mass range is shown on the x-axis, resolution was set to 0.1 Da/channel; a simulated isotope pattern model was used as damage model, while the blur width was determined via the peak width at half the of the peak height of the most abundant peak; the deconvolution was iterated until convergence and the mock results were compared with the initial mass spectra to ensure success of the deconvolution.