Supplemental Material for the Manuscript:

Structural characterization of CAS SH3 domain selectivity and regulation reveals new CAS interaction partners

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Supplemental figures and tables

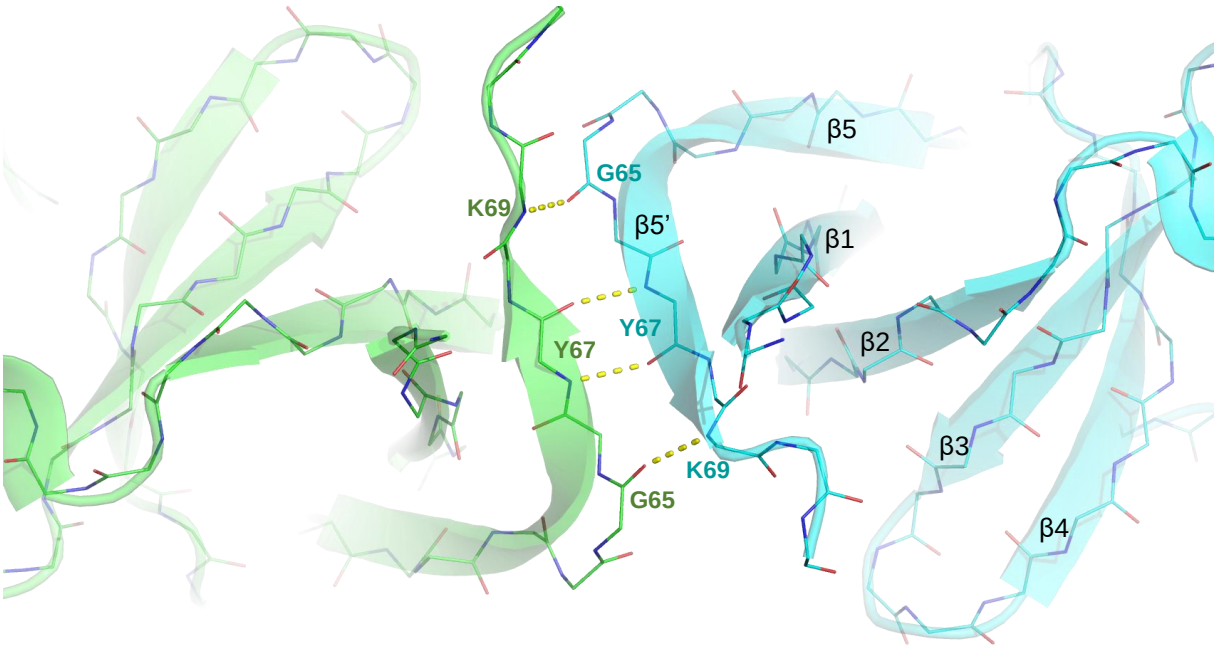


Figure S1. The crystallographic dimer (PDB code 1WYX) is stabilized by the crystal contacts that stimulate the formation of an additional two-stranded (labeled b5') beta-sheet at the dimer interface. The beta-sheet is stabilized by a network of four intra-main chain hydrogen bonds (highlighted in yellow).

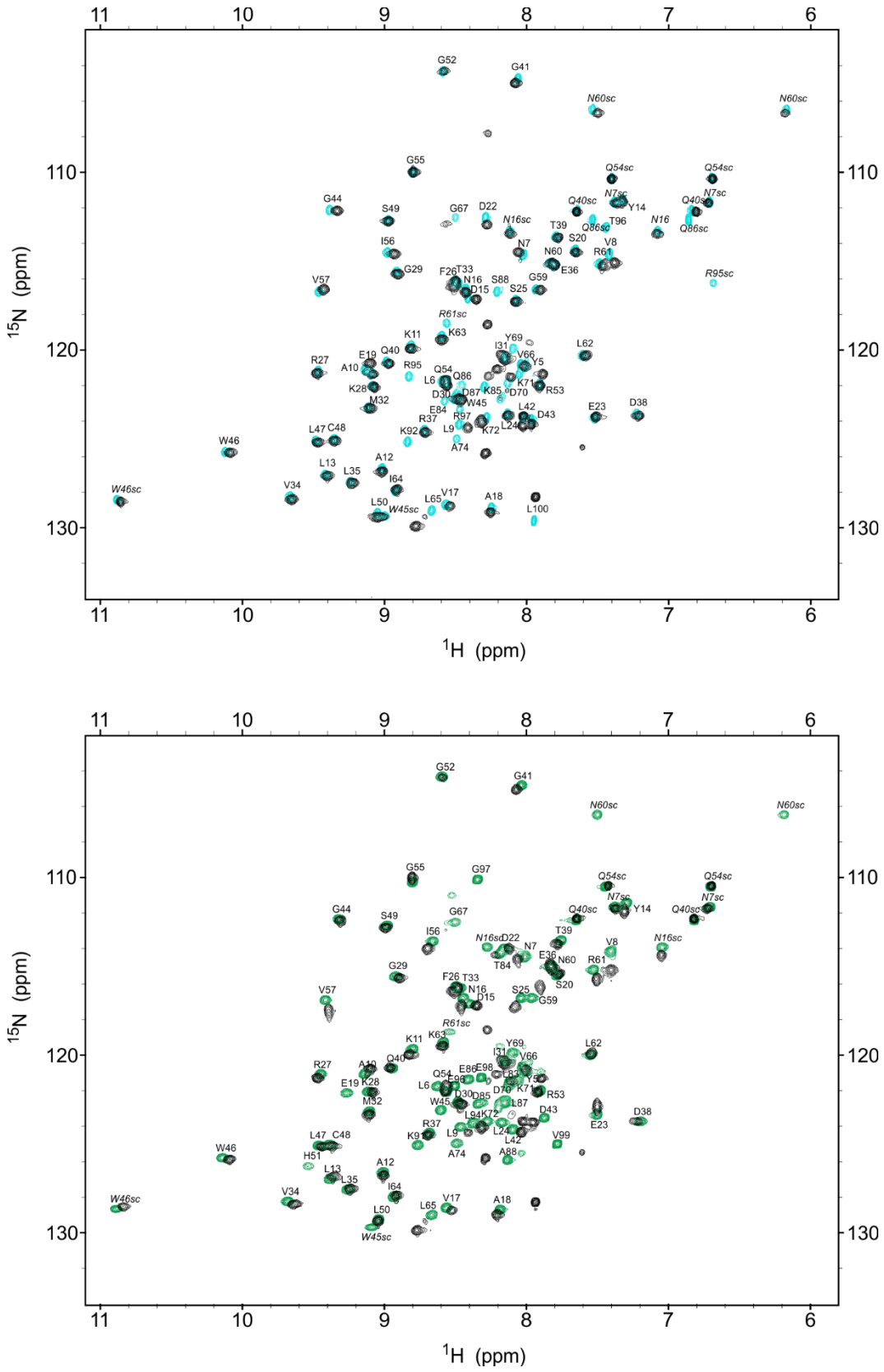


Figure S2. Overlays of ^{15}N/^{1}N HSQC spectra of the free the CAS SH3 domain with bound peptide from PTP-PEST or Vinculin (black) and PTP-PEST (cyan) and Vinculin (green) chimeras.

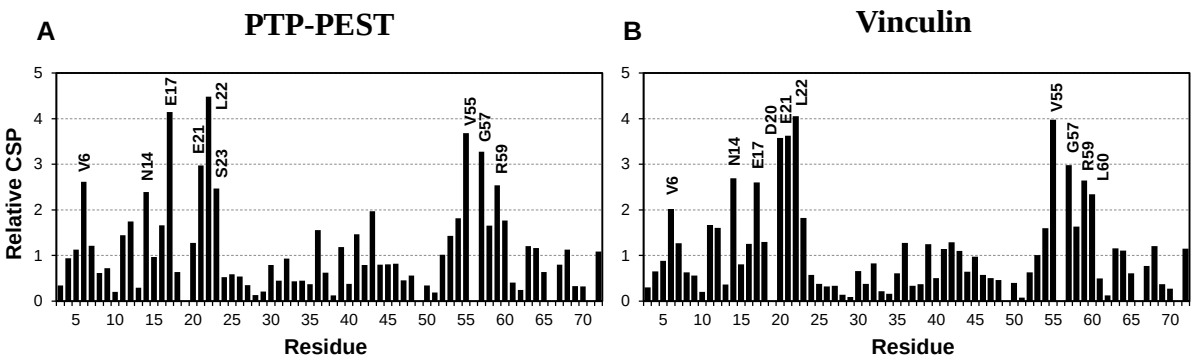


Figure S3. Relative chemical shift perturbations (CSP) of CAS SH3 induced by binding of **A** PTP-PEST peptide and **B** Vinculin peptide. The values were calculated from changes in positions of backbone amide resonances and divided by standard deviation. The distribution of induced changes is similar for both peptides, corresponding to highly overlapping binding regions derived from the structural data obtained for both complexes.

A

```
                    Position
                  …-10123456789…
FAK/FRNKb         PDPAAPPKKPPRPGAPGH
PYK2b             LEFTGPPQKPPRLGAQSI
C3G               TDEEVAPPKPPLPGIRVV
Vinculin          LTDELAPPKPPLPEGEVP
FAK/FRNKa         GGSDEAPPKPSRPGYPSP
PYK2a             TAFQEPPPKPSRPKYRPP
PTP-PEST          EKQDSPPPKPPRTRSCLV
MICA1             DPEMEPPPKPPRSCSALA
                        * **
```

B

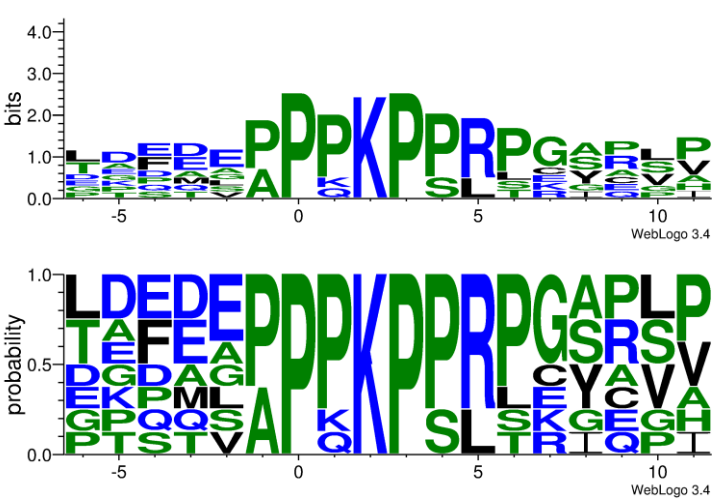


Figure S4. Enrichment of negatively charged amino acids within CAS SH3 binding ligands. **A** Multiple sequence alignment of eight high-affinity CAS SH3 binding sequences derived from known six human CAS SH3 binding proteins using CLUSTAL W. **B** Prolonged high-affinity CAS SH3 binding motif derived from sequences belonging to known human CAS SH3 interacting proteins**.** The x -axis shows the positions in relation to proline (position 0). The y-axis in the upper panel shows the bits. The graphs show the amino acid preference near the proline. The height of the letters is proportional to the frequency in the top eight protein sequences.

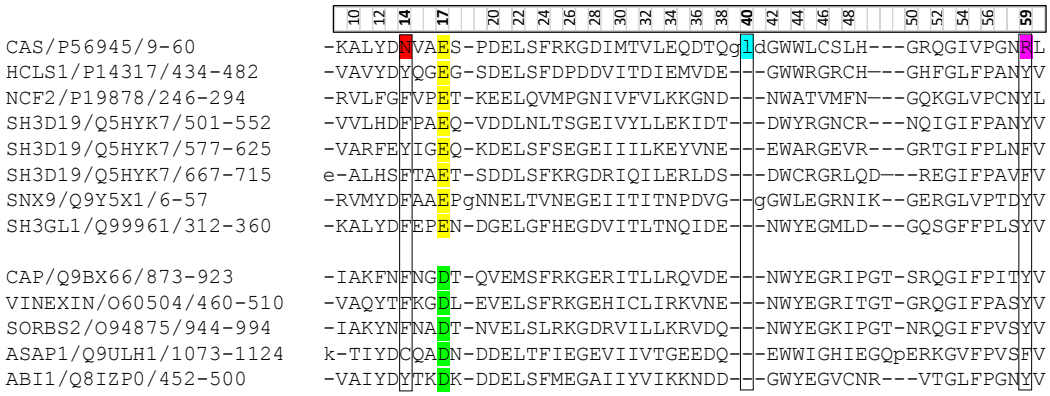
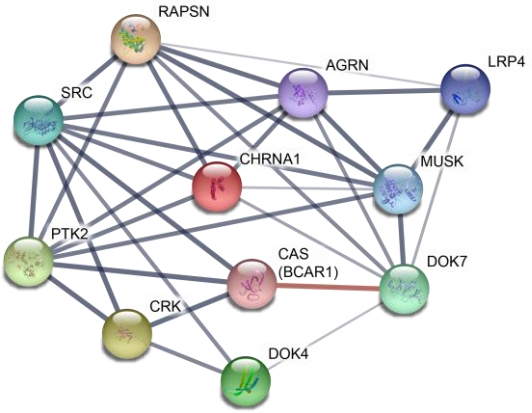


Figure S5. Alignment of human SH3 domains that are likely to bind a centrally located lysine in the ligand. Numbers indicate the primary amino acid sequence of the CAS SH3 domain. The alignment includes 13 unique SH3 domains from 11 proteins that include either Glu (yellow column) or Asp (green column) at the position equivalent to Glu17 in CAS protein and a conserved Glu that corresponds to CAS Glu21 (orange column). Unique sequence features of CAS, namely Asn14, Leu40 and Arg59, are also highlighted. Amino acids in small letters indicate positions in the alignment that are occupied in a small proportion of sequences.

A

Prot. name	Sequence	Interaction with SH3 p130Cas
C15oRf62	A_{78}PP**KP**PRL	?
C1oRf168	P_{278}PP**KP**SRP	?
CEP2	A_{126}PP**KP**PRL	?
DOK7	P_{317}PP**KP**LRP	?
FBN2	P_{33}PP**KP**PRP	?
GLIS2	P_{333}PP**KP**PLP	?
MICAL1	P_{829}PP**KP**PRS	Known
PKN3	P_{509}PP**KP**PRL	?
PRAM1	P_{501}PA**KP**PLP	?
FAK	A_{711}PP**KP**SRP	Known
PYK2	P_{713}PP**KP**SRP	Known
PTP-PEST	P_{333}PP**KP**PRT	Known
C3G	A_{266}PP**KP**PLP	Known
SETD5	P_{606}PA**KP**SRP	?
Vinculin	A_{859}PP**KP**PLP	Known
WNK1	A_{1750}PS**KP**PLT	?

B



C

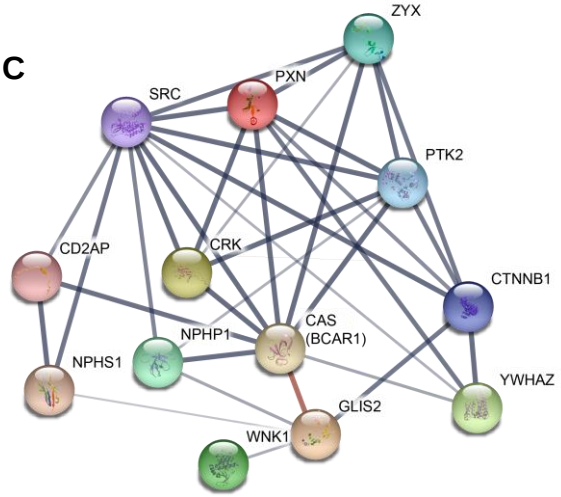


Figure S6. Potential CAS/BCAR1 interaction partners. **A** Table of known and predicted CAS SH3 interacting proteins based on CAS SH3 binding motif. Six have already been shown to bind CAS. In proteins highlighted by gray boxes, we were able to find connections to the signaling processes involving CAS. **B** Signaling network of proteins that are important for acetylcholine receptor clustering in skeletal muscle with added CAS. The red line represents the new **CAS-DOK7** interaction. **C** Signaling network of proteins implicated in regulation of kidney function. The red line represents the new **CAS-GLIS2** interaction. Crosstalk is illustrated with STRING 10 (http://version_10.string-db.org/). Line thickness indicates the strength of data support, a red line represents a newly verified interaction.

CAS_SH3

Amino acid sequence after thrombin cleavage:

GSMKYLNVLAKALYDNVAESPDELSFRKGDIMTVLERDTQGLDGWWLCSLHGRQGIVPGNRLKILVGMYDKKPAG*EFIVTD*

CAS_SH3_Vinculin

Amino acid sequence after thrombin cleavage:

GSMKYLNVLAKALYDNVAESPDELSFRKGDIMTVLERDTQGLDGWWLCSLHGRQGIVPGNRLKILVGMYDKKPAGSGGSGS
GLTDELAPPKPPLPEGEV

CAS_SH3_PTP-PEST

Amino acid sequence after thrombin cleavage:

GSMKYLNVLAKALYDNVAESPDELSFRKGDIMTVLERDTQGLDGWWLCSLHGRQGIVPGNRLKILVGMYDKKPAGSGGSGS
GSEKQDSPPPKPPRTRSCL

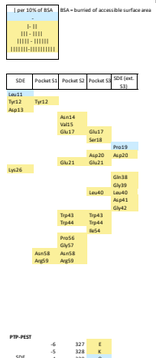
Figure S7. Primary sequences of the CAS SH3 domain chimeras. The first two amino acids (GS) are residuals from the thrombin cleavage site. Blue indicates the extended sequence from cloning vector ended by introduction of a stop codon from *Eco*RI restriction site. Red represents the inserted sequence of the linker. Sequences from the ligands are underlined.

Supplementary table 1. NMR restraints and structural statistics

	SH3	*PTP-PEST*	*Vinculin*
Non-redundant distance and angle constrains			
Total number of NOE constraints	1165	1998	2483
Short-range NOEs			
Intra-residue (i = j)	208	426	494
Sequential (| i - j | = 1)	305	468	585
Medium-range NOEs (1 < | i - j | < 5)	122	183	267
Long-range NOEs (| i - j | ≥ 5)	530	921	1137
Torsion angles	100	80	88
Hydrogen bond restrains	-	-	-
Total number of restricting constraints	1265	2078	2571
Total restricting constraints per restrained residue	17.6	23.9	28.9
Residual constraint violations			
Distance violations per structure			
0.1 – 0.2 Å	3.45	9.25	8.40
0.2 – 0.5 Å	2.22	3.65	3.80
> 0.5 Å	0	0	0
r.m.s. of distance violation per constraint	0.02 Å	0.02 Å	0.02 Å
Maximum distance violation	0.50 Å	0.50 Å	0.50 Å
Dihedral angle violations per structure			
1 – 10 °	2.33	1.23	0.9
> 10 °	0	0	0
r.m.s. of dihedral violations per constraint	0.51 °	0.46 °	0.31 °
Maximum dihedral angle violation	5.00 °	5.00 °	4.90 °
Ramachandran plot summary from Procheck			
Most favoured regions	96.4%	92.3%	93.1%
Additionally allowed regions	3.6%	7.5%	6.7%
Generously allowed regions	0.0%	0.1%	0.1%
Disallowed regions	0.0%	0.0%	0.0%

r.m.s.d. to the mean structure	*ordered*[1]	*all residues*	*ordered*[1]	*all residues*	*ordered*[1]	*all residues*
All backbone atoms	0.3 Å	6.7 Å	0.3 Å	2.7 Å	0.2 Å	2.4 Å
All heavy atoms	0.9 Å	6.9 Å	0.7 Å	3.0 Å	0.5 Å	2.6 Å

[1] Residues with sum of phi and psi order parameters > 1.8



	Numbering from	CAS SH3-(PTP-PEST)	01 PEST	02 PEST	03 PEST	04 PEST	05 PEST	06 PEST	07 PEST	08 PEST	09 PEST	10 PEST	11 PEST	12 PEST	13 PEST	14 PEST	15 PEST	16 PEST	17 PEST	18 PEST	19 PEST	20 PEST	21 PEST	22 PEST	23 PEST	24 PEST	25 PEST	26 PEST	27 PEST	28 PEST	29 PEST	30 PEST	31 PEST	32 PEST	33 PEST	34 PEST	35 PEST	36 PEST	37 PEST	38 PEST	39 PEST	40 PEST
	UNIPROT	UNIPROT	BSA/I BIG PHOC																																							
PEST	1	Met 3																																							
	2	Lys 4																																							
	3	Tyr5																																							
	4	Leu 6																																							
	5	Asn 7																																							
	6	Val 8																																							
	7	Leu 9																																							
	8	Ala 10																																							
	9	Lys 11																																							
	10	Ala 12																																							
	11	Leu 13																																							
	12	Tyr 14																																							
	13	Asp 15																																							
	14	Asn 16																																							
	15	Val 17																																							
	16	Ala 18																																							
	17	Glu 19																																							
	18	Ser 20																																							
	19	Pro 21																																							
	20	Asp 22																																							
	21	Glu 23																																							
	22	Leu 24																																							
	23	Ser 25																																							
	24	Phe 26																																							
	25	Arg 27																																							
	26	Lys 28																																							
	27	Gly 29																																							
	28	Asp 30																																							
	29	Ile 31																																							
	30	Met 32																																							
	31	Thr 33																																							
	32	Val 34																																							
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	34	Glu 36																																							
	35	Arg 37																																							
	36	Asp 38																																							
	37	Thr 39																																							
	38	Gln 40																																							
	39	Gly 41																																							
	40	Leu 42																																							
	41	Asp 43																																							
	42	Gly 44																																							
	43	Trp 45																																							
	44	Trp 46																																							
	45	Leu 47																																							
	46	Cys 48																																							
	47	Ser 49																																							
	48	Leu 50																																							
	49	His 51																																							
	50	Gly 52																																							
	51	Arg 53																																							
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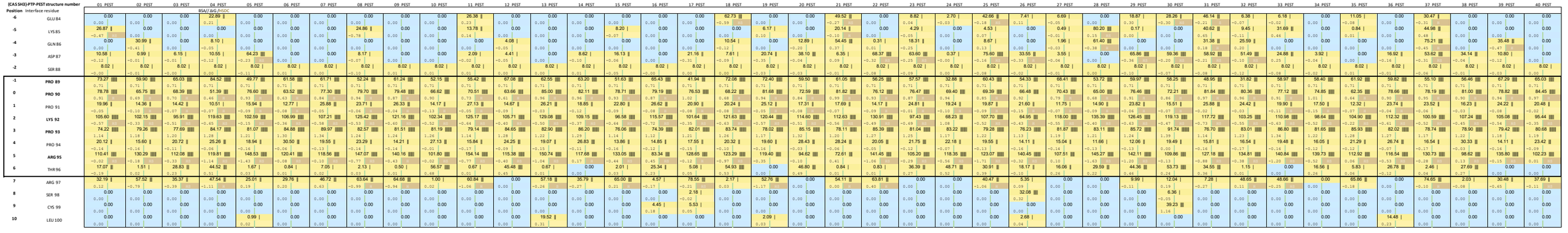
	65	Gly 67																																							
	66	Met 68																																							
	67	Tyr 69																																							
	68	Asp 70																																							
	69	Lys 71																																							
	70	Lys 72																																							
	71	Pro 73																																							
	72	Ala 74																																							

Supplementary table S2. Interface analysis of CAS SH3-PTP-PEST (SH3 part).
Hydrogen bonds and nonpolar contacts between Cas SH3 and its ligands were analyzed with PISA with default settings. Prior to analysis, the linker sequence (residues 75-82) was removed from the chimeric proteins. Solvent accessible residues are colored yellow and inaccessible residues are blue. ΔiG represents free energy of dissociation (solvation energy effect, kcal/mol). BSA means Buried Surface Area (Å²) and is displayed with bars, one bar per 10%. HSDC indicates residues that contain the across-interface hydrogen bond, salt bridge, or disulfide bond atoms. The corresponding table cells have a brown background and contain letter H for a hydrogen-bond, S for a salt bridge, and HS for a combination of hydrogen bond and salt bridge.
http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html



Supplementary table S3. Interface analysis of CAS SH3-PTP-PEST (ligand part).
Hydrogen bonds and nonpolar contacts between Cas SH3 and its ligands were analyzed with PISA with default settings. Prior to analysis, the linker sequence (residues 75-82) was removed from the chimeric proteins. Solvent accessible residues are colored yellow and inaccessible residues are blue. ΔiG represents free energy of dissociation (solvation energy effect, kcal/mol). BSA means Buried Surface Area (Å^2) and is displayed with bars, one bar per 10%. HSDC indicates residues that contain the across-interface hydrogen bond, salt bridge, or disulfide bond atoms. The corresponding table cells have a brown background and contain letter H for a hydrogen bond, S for a salt bridge, and HS for a combination of hydrogen bond and salt bridge.
http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html

Hydrogen bonds (Interface) XML

##	UNIPROT	CAS SH3	PTP-PEST		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	SDE	Pocket S1	Pocket S2	Pocket S3	SDE (ext. S3)
					Structure (number)																																												
1	Lys26	A:LYS 28[NZ]	B:GLU 84[OE1]	-6																		X												X											Lys26				
2		A:LYS 28[NZ]	B:GLU 84[OE2]	-6																																													
3	Arg25	A:ARG 27[NH1]	B:GLU 84[OE1]	-6																									X																Arg25				
4		A:ARG 27[NH1]	B:GLU 84[OE2]	-6																					X																								
5		A:ARG 27[NH2]	B:GLU 84[OE2]	-6																									X																				
6		A:ARG 27[HE]	B:GLU 84[O]	-6																																					X				Arg25				
7		A:ARG 27[NH2]	B:GLU 84[O]	-6																																					X								
8	Arg25	A:ARG 27[NH2]	B:LYS 85[O]	-5																					X																				Arg25				
9	Asp13	A:ASP 15[OD1]	B:LYS 85[NZ]	-5																																									Asp13				
10	Asp13	A:ASP 15[OD1]	B:LYS 85[NZ]	-5	X																											X													Asp13				
11	Asp13	A:ASP 15[OD2]	B:GLN 86[H]	-4																																					X				Asp13				
12		A:ASP 15[OD1]	B:GLN 86[H]	-4																															X														
13	Asp13	A:ASP 15[OD1]	B:GLN 86[NE2]	-4																																					X				Asp13				
14	Lys26	A:LYS 28[NZ]	B:GLN 86[OE1]	-4																												X											X		Lys26				
15	Lys26	A:LYS 28[NZ]	B:ASP 87[OD1]	-3					X																		X		X				X		X						X				Lys26				
16		A:LYS 28[NZ]	B:ASP 87[OD2]	-3																						X								X	X	X													
17	Tyr12	A:TYR 14[HH]	B:ASP 87[OD2]	-3																										X															Tyr12	Tyr12			
18	Arg59	A:ARG 61[NH2]	B:PRO 90[O]	0	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X		X		X	X	X	X	X	X	X		X	X	X	X	X	X		Arg59	Arg59		
19	Arg59	A:ARG 61[HE]	B:PRO 90[O]	0	X						X						X																		X							X				Arg59	Arg59		
20	Asn58	A:ASN 60[ND2]	B:PRO 90[O]	0		X	X	X		X						X															X	X							X							Asn58	Asn58		
21	Asn58	A:ASN 60[ND2]	B:PRO 91[O]	1		X	X	X	X	X		X	X	X	X					X	X	X	X	X		X	X	X	X	X	X	X	X	X			X	X	X	X						Asn58	Asn58		
22	Glu17	A:GLU 19[OE1]	B:LYS 92[NZ]	2		X	X			X			X		X	X										X							X	X				X					X				Glu17	Glu17	
23		A:GLU 19[OE2]	B:LYS 92[NZ]	2	X							X		X			X												X			X			X	X						X					Glu17	Glu17	
24	Val15	A:VAL 17[O]	B:LYS 92[NZ]	2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			Val15		
26	Glu21	A:GLU 23[OE1]	B:LYS 92[NZ]	2			X	X		X	X	X		X	X	X			X				X			X	X				X	X		X				X		X			X				Glu21	Glu21	
27		A:GLU 23[OE2]	B:LYS 92[NZ]	2	X	X		X	X			X	X	X	X		X	X		X	X	X		X	X	X	X		X		X		X	X		X	X		X	X	X	X		X			Glu21	Glu21	
28	Asn14	A:ASN 16[OD1]	B:LYS 92[NZ]	2																										X																	Asn14		
29	Glu21	A:GLU 23[OE1]	B:ARG 95[NH1]	5	X				X		X			X															X							X	X				X						Glu21	Glu21	
30		A:GLU 23[OE1]	B:ARG 95[NH2]	5		X																								X									X										
31		A:GLU 23[OE2]	B:ARG 95[NH1]	5			X			X						X			X								X											X				X	X						
32		A:GLU 23[OE2]	B:ARG 95[NH2]	5												X														X														X					
33	Glu17	A:GLU 19[OE1]	B:ARG 95[NH1]	5										X						X																				X			X				Glu17	Glu17	
34		A:GLU 19[OE1]	B:ARG 95[NH2]	5		X				X				X																								X					X						
35		A:GLU 19[OE2]	B:ARG 95[NH1]	5	X																								X							X													
36		A:GLU 19[OE2]	B:ARG 95[NH2]	5	X		X																													X													
37	Ser18	A:SER 20[OG]	B:ARG 95[HE]	5															X																	X												Ser18	
38	Ser18	A:SER 20[OG]	B:ARG 95[NH1]	5	X			X		X			X			X		X			X					X	X		X			X					X											Ser18	
39		A:SER 20[OG]	B:ARG 95[NH2]	5					X		X		X								X																												
40	Asp20	A:ASP 22[OD1]	B:ARG 95[NH1]	5			X	X																																								Asp20	Asp20
41		A:ASP 22[OD1]	B:ARG 95[NH2]	5				X							X		X											X				X			X														
42		A:ASP 22[OD2]	B:ARG 95[NH1]	5																																													
43		A:ASP 22[OD2]	B:ARG 95[NH2]	5								X										X				X							X																
44	Asp20	A:ASP 22[OD1]	B:ARG 97[NH1]	7			X																																										
45	Gly39	A:GLY 41[O]	B:ARG 97[NH2]	7								X			X										X																								Gly39
46		A:GLY 41[O]	B:ARG 97[NH1]	7									X						X							X										X					X								
47	Gln38	A:GLN 40[OE1]	B:ARG 97[NH2]	7				X																					X				X																Gln38
48	Asp41	A:ASP 43[OD1]	B:ARG 97[NH1]	7																																	X												Asp41
49		A:ASP 43[OD1]	B:ARG 97[NH2]	7																	X																												
50		A:ASP 43[OD2]	B:ARG 97[NH1]	7																			X																					X					
51		A:ASP 43[OD2]	B:ARG 97[NH2]	7																																													

Salt bridges (Interface) XML

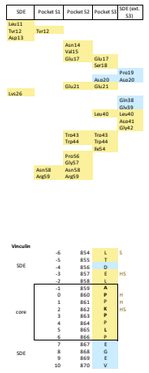
##	UNIPROT	CAS SH3	PTP-PEST		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	SDE	Pocket S1	Pocket S2	Pocket S3	SDE (ext. S3)
					Structure (number)																																												
1	Lys26	A:LYS 28[NZ]	B:GLU 84[OE1]	-6																		X												X											Lys26				
2		A:LYS 28[NZ]	B:GLU 84[OE2]	-6																		X																											
3	Arg25	A:ARG 27[NH1]	B:GLU 84[OE1]	-6																									X																Arg25				
4		A:ARG 27[NH2]	B:GLU 84[OE1]	-6																									X																				
5		A:ARG 27[NH1]	B:GLU 84[OE2]	-6																					X				X																				
6		A:ARG 27[NH2]	B:GLU 84[OE2]	-6																					X				X						X														
7	Asp13	A:ASP 15[OD1]	B:LYS 85[NZ]	-5	X																											X													Asp13				
8	Lys26	A:LYS 28[NZ]	B:ASP 87[OD1]	-3					X																		X		X				X	X	X	X					X				Lys26				
9		A:LYS 28[NZ]	B:ASP 87[OD2]	-3					X																	X			X					X	X														
10	Glu21	A:GLU 23[OE1]	B:LYS 92[NZ]	2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X			Glu21	Glu21	
11		A:GLU 23[OE2]	B:LYS 92[NZ]	2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X					
12	Glu17	A:GLU 19[OE1]	B:LYS 92[NZ]	2			X			X			X		X	X				X						X							X	X	X	X		X					X				Glu17	Glu17	
13		A:GLU 19[OE2]	B:LYS 92[NZ]	2	X	X						X		X			X			X			X						X			X			X	X					X	X							
14	Glu21	A:GLU 23[OE1]	B:ARG 95[NE]	5		X																								X									X								Glu21	Glu21	
15		A:GLU 23[OE2]	B:ARG 95[NE]	5																										X														X					
16	Glu21	A:GLU 23[OE1]	B:ARG 95[NH1]	5	X		X			X	X																		X							X	X				X		X				Glu21	Glu21	
17		A:GLU 23[OE1]	B:ARG 95[NH2]	5		X								X		X													X	X								X	X					X					
18		A:GLU 23[OE2]	B:ARG 95[NH1]	5			X		X							X			X								X											X				X	X						
19		A:GLU 23[OE2]	B:ARG 95[NH2]	5		X										X														X								X	X					X					
20	Glu17	A:GLU 19[OE2]	B:ARG 95[NE]	5																										X																	Glu17	Glu17	
21	Glu17	A:GLU 19[OE1]	B:ARG 95[NH1]	5		X	X			X										X										X										X			X				Glu17	Glu17	
22		A:GLU 19[OE1]	B:ARG 95[NH2]	5		X	X			X																				X								X		X			X						
23		A:GLU 19[OE2]	B:ARG 95[NH1]	5	X									X															X	X						X				X									
24		A:GLU 19[OE2]	B:ARG 95[NH2]	5	X									X																						X				X									
25	Asp20	A:ASP 22[OD1]	B:ARG 95[NH1]	5				X							X		X					X																										Asp20	Asp20
26		A:ASP 22[OD1]	B:ARG 95[NH2]	5				X							X		X					X				X		X				X	X		X														
27		A:ASP 22[OD2]	B:ARG 95[NH1]	5																													X																
28		A:ASP 22[OD2]	B:ARG 95[NH2]	5																													X																
29	Asp20	A:ASP 22[OD1]	B:ARG 97[NH1]	7			X																																									Asp20	Asp20
30	Asp41	A:ASP 43[OD1]	B:ARG 97[NH1]	7													X																				X											Asp41	Asp41
31		A:ASP 43[OD1]	B:ARG 97[NH2]	7													X				X																												
32		A:ASP 43[OD2]	B:ARG 97[NH1]	7													X						X																					X					

SH3 numbering from UNIPROT: X-2

number of structures
0-5 %
6-20 %
21-50 %
51-80%
81-100%

	PTP-PEST		structure	UNIPROT
SDE	-6	E	84	327
	-5	K	85	328
	-4	Q	86	329
	-3	D	87	330
	-2	S	88	331
core	-1	**P**	89	332
	0	**P**	90	333
	1	P	91	334
	2	**K**	92	335
	3	**P**	93	336
	4	P	94	337
	5	**R**	95	338
	6	T	96	339
SDE	7	R	97	340
	8	S	98	341
	9	C	99	342
	10	L	100	343

Supplementary table S4. Interface analysis of CAS SH3-PTP-PEST (hydrogen bonds).
Hydrogen bonds and salt bridges between Cas SH3 and its ligands were analyzed with PISA with default settings. Prior to analysis, the linker sequence (residues 75-82) was removed from the chimeric proteins. The atoms that form hydrogen bonds and salt bridges are indicated by crosses.

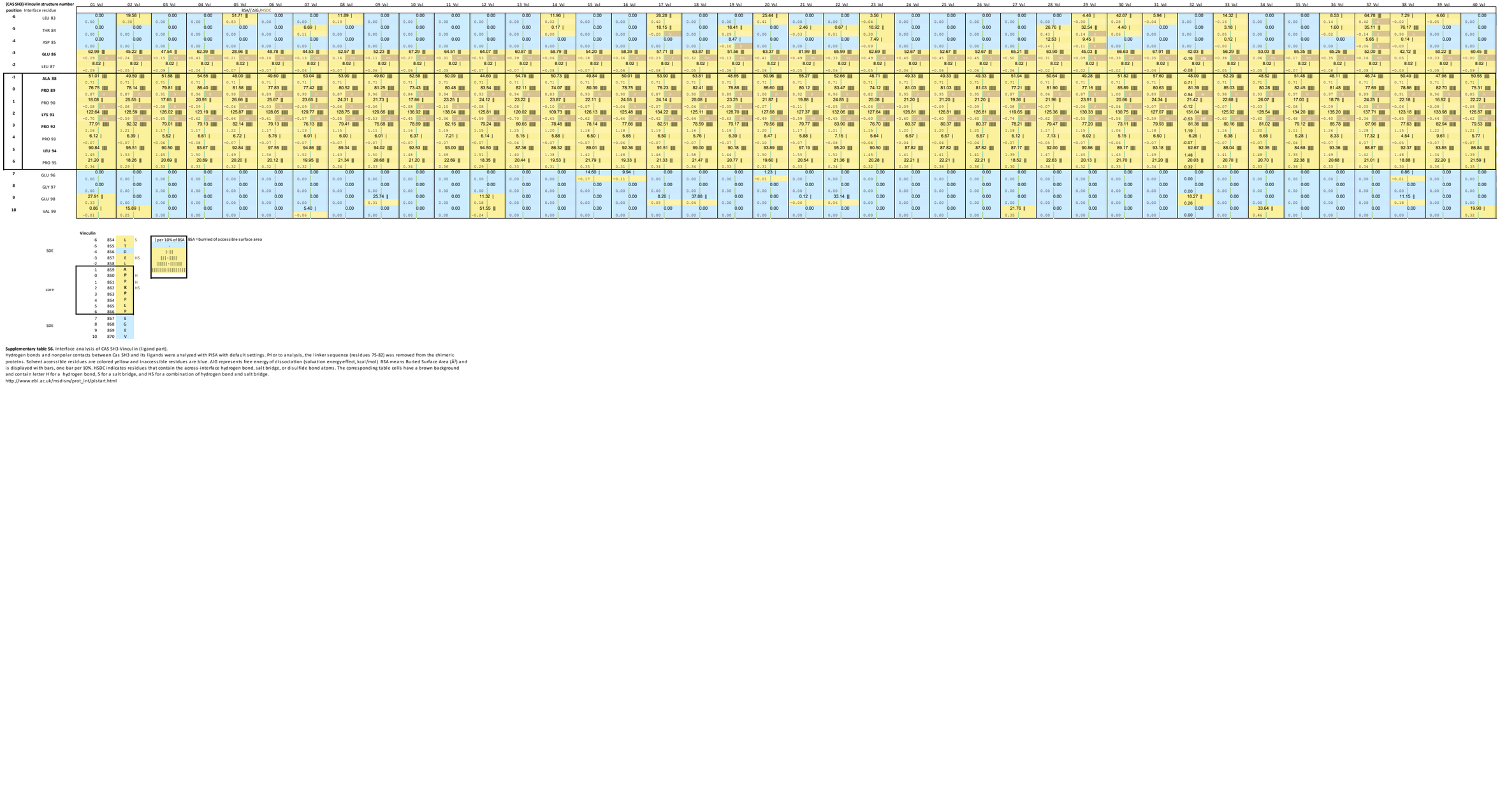


Vinculin

	-6	854	L	S
	-5	855	T	
SDE	-4	856	G	D
	-3	857	E	HS
	-2	858	L	
	-1	859	A	
	0	860	P	H
	1	861	P	H
core	2	862	K	HS
	3	863	P	
	4	864	T	
	5	865	L	
	6	866	P	
	7	867	E	
SDE	8	868	G	
	9	869	E	
	10	870	V	

Numbering from UNIPROT:	CAS SH3 (Vinculin) structure	01_Val	02_Val	03_Val	04_Val	05_Val	06_Val	07_Val	08_Val	09_Val	10_Val	11_Val	12_Val	13_Val	14_Val	15_Val	16_Val	17_Val	18_Val	19_Val	20_Val	21_Val	22_Val	23_Val	24_Val	25_Val	26_Val	27_Val	28_Val	29_Val	30_Val	31_Val	32_Val	33_Val	34_Val	35_Val	36_Val	37_Val	38_Val	39_Val	40_Val
		BSA(Å²)	BSA(Å²)	PHOC																																					
Vinculin	Interface residue																																								
1	Met 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	Lys 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	Tyr5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	Leu 6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	Asn 7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	Val 8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	Leu 9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	Ala 10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	Lys 11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	Ala 12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	Leu 13	0.00	0.00	0.12	0.74	0.00	0.00	0.00	0.00	2.21	0.00	1.72	0.00	0.81	1.27	0.00	0.00	1.11	0.46	2.18	0.81	0.98	2.46	0.49	0.37	0.91	0.41	0.41	0.40	0.74	0.00	0.74	0.37	0.00	0.25	0.12	1.11	0.00	2.06	0.98	0.00
12	Tyr 14	65.62	74.67	67.29	70.09	71.13	72.19	67.73	75.31	74.72	75.37	71.49	76.24	68.46	80.75	67.00	74.74	77.07	73.86	75.77	75.64	79.31	74.19	81.47	73.51	73.51	73.51	70.40	77.66	83.15	71.39	73.08	63.75	71.21	78.06	78.17	77.18	84.47	78.92	67.84	65.36
13	Asp 15	0.98	1.99	2.95	4.30	23.59	2.25	2.94	3.44	3.14	2.21	3.02	1.57	3.48	6.05	3.26	3.22	7.86	3.01	2.16	3.69	6.58	5.15	3.58	2.20	2.20	2.20	0.29	5.27	2.01	2.33	4.18	3.60	3.32	3.14	2.16	1.40	27.61	6.86	1.40	2.21
14	Asn 16	-0.03 5.04	4.17	-0.03 4.54	-0.03 4.91	-0.03 3.92	-0.03 1.59	-0.03 0.49	-0.03 6.14	-0.03 2.70	-0.03 1.96	-0.03 2.33	-0.03 4.91	-0.03 5.40	-0.03 1.35	-0.03 0.98	-0.03 2.58	-0.03 4.29	-0.03 6.63	-0.03 4.30	-0.03 5.15	-0.03 4.05	-0.03 5.15	-0.03 1.10	-0.03 2.45	-0.03 2.45	-0.03 2.45	-0.03 2.04	-0.03 3.07	-0.03 5.03	-0.03 5.28	-0.03 5.40	-0.03 0.98	-0.03 5.16	-0.03 1.35	-0.03 2.94	-0.03 1.74	-0.03 2.45	-0.03 1.10	-0.03 4.78	-0.03 0.73
15	Val 17	-0.04 3.31	3.21	-0.04 3.58	-0.04 3.22	-0.04 3.44	-0.04 2.46	-0.04 1.72	-0.04 2.72	-0.04 1.96	-0.04 1.72	-0.04 3.19	-0.04 1.59	-0.04 3.95	-0.04 0.98	-0.04 1.96	-0.04 3.32	-0.04 4.49	-0.04 3.71	-0.04 2.14	-0.04 4.80	-0.04 3.46	-0.04 4.12	-0.04 1.84	-0.04 3.56	-0.04 3.56	-0.04 3.56	-0.04 3.56	-0.04 2.36	-0.04 2.36	-0.04 1.23	-0.04 2.73	-0.04 2.45	-0.04 2.16	-0.04 3.19	-0.04 2.48	-0.04 6.15	-0.04 3.58	-0.04 1.10	-0.04 3.83	-0.04 3.74
16	Ala 18	24.96	26.21	24.86	25.53	25.21	24.57	24.81	25.19	25.19	26.01	23.86	26.38	25.35	24.53	25.64	26.01	22.98	25.52	25.64	21.02	25.26	20.98	26.23	26.41	26.41	26.41	25.29	23.74	25.79	25.71	25.09	27.38	24.60	24.12	25.52	23.48	22.95	25.76	23.08	25.43
17	Glu 19	-0.26 21.87	22.24	-0.26 19.65	-0.26 19.15	-0.26 21.58	-0.26 22.25	-0.26 18.08	-0.26 19.83	-0.26 19.41	-0.26 19.36	-0.26 20.45	-0.26 19.45	-0.26 18.32	-0.26 18.59	-0.26 19.31	-0.26 18.67	-0.26 19.66	-0.26 21.97	-0.26 18.14	-0.26 21.03	-0.26 21.81	-0.26 20.05	-0.26 19.49	-0.26 21.92	-0.26 21.92	-0.26 21.92	-0.26 20.06	-0.26 20.04	-0.26 18.19	-0.26 19.79	-0.26 21.81	-0.26 19.41	-0.26 20.16	-0.26 17.21	-0.26 19.11	-0.26 21.57	-0.26 18.30	-0.26 21.69	-0.26 17.39	-0.26 19.47
18	Pro 21	-0.16 5.86	0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 13.72	-0.16 3.87	-0.16 0.00	-0.16 18.57	-0.16 0.00	-0.16 1.51	-0.16 5.17	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 0.00	-0.16 2.75	-0.16 0.00	-0.16 37.66	-0.16 0.00	-0.16 0.00	-0.16 1.01	-0.16 0.00	-0.16 0.00
19	Asp 22	12.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.86	0.00	0.00	5.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	Glu 23	-0.14 8.13	6.30	-0.14 5.10	-0.14 5.22	-0.14 6.26	-0.14 8.01	-0.14 7.98	-0.14 7.77	-0.14 6.79	-0.14 6.05	-0.14 6.26	-0.14 6.22	-0.14 5.50	-0.14 7.64	-0.14 6.26	-0.14 7.28	-0.14 6.26	-0.14 6.78	-0.14 7.64	-0.14 6.26	-0.14 6.26	-0.14 6.26	-0.14 6.26	-0.14 6.26	-0.14 6.26	-0.14 6.26	-0.14 10.06	-0.14 6.47	-0.14 6.36	-0.14 9.48	-0.14 8.72	-0.14 7.24	-0.14 7.98	-0.14 7.52	-0.14 8.62	-0.14 8.38	-0.14 7.17	-0.14 7.77	-0.14 7.64	-0.14 8.72
21	Leu 24	-0.08 40.99	51.09	-0.08 34.55	-0.08 30.26	-0.08 38.00	-0.08 32.67	-0.08 33.87	-0.08 47.53	-0.08 32.10	-0.08 32.81	-0.08 31.58	-0.08 33.26	-0.08 38.64	-0.08 40.93	-0.08 39.84	-0.08 31.56	-0.08 66.40	-0.08 29.11	-0.08 44.08	-0.08 33.63	-0.08 35.63	-0.08 30.84	-0.08 38.19	-0.08 29.62	-0.08 29.62	-0.08 29.62	-0.08 30.79	-0.08 37.65	-0.08 48.56	-0.08 48.34	-0.08 24.25	-0.08 40.03	-0.08 30.88	-0.08 33.66	-0.08 35.21	-0.08 38.67	-0.08 66.35	-0.08 45.94	-0.08 21.53	-0.08 43.36
22	Ser 25	-0.14 10.60	12.20	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 5.28	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 5.15	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 18.24	-0.14 0.00	-0.14 14.99	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 0.00	-0.14 24.08	-0.14 0.00
23	Phe 26	-0.18 41.75	44.94	-0.18 41.74	-0.18 40.75	-0.18 42.11	-0.18 40.57	-0.18 39.57	-0.18 42.18	-0.18 42.76	-0.18 40.05	-0.18 41.74	-0.18 47.29	-0.18 39.20	-0.18 40.74	-0.18 40.55	-0.18 37.73	-0.18 42.59	-0.18 39.56	-0.18 42.06	-0.18 38.71	-0.18 40.90	-0.18 42.66	-0.18 38.87	-0.18 42.10	-0.18 42.10	-0.18 42.10	-0.18 42.08	-0.18 41.08	-0.18 40.22	-0.18 40.41	-0.18 40.39	-0.18 41.40	-0.18 42.25	-0.18 41.94	-0.18 44.45	-0.18 41.27	-0.18 41.73	-0.18 39.07	-0.18 42.27	-0.18 42.61
24	Arg 27	0.87 10.59	12.64	-0.87 11.57	-0.87 8.96	-0.87 12.03	-0.87 11.01	-0.87 11.91	-0.87 10.94	-0.87 12.77	-0.87 11.31	-0.87 12.40	-0.87 11.16	-0.87 12.27	-0.87 11.17	-0.87 12.94	-0.87 12.27	-0.87 10.80	-0.87 11.30	-0.87 12.41	-0.87 12.02	-0.87 10.32	-0.87 12.26	-0.87 10.67	-0.87 12.74	-0.87 12.74	-0.87 12.74	-0.87 11.05	-0.87 13.14	-0.87 11.78	-0.87 8.95	-0.87 12.29	-0.87 12.03	-0.87 12.88	-0.87 12.27	-0.87 11.92	-0.87 12.78	-0.87 8.95	-0.87 22.04	-0.87 12.64	-0.87 13.38
25	Leu 28	-0.12 10.68	11.36	-0.12 11.14	-0.12 13.69	-0.12 11.41	-0.12 10.82	-0.12 11.42	-0.12 11.42	-0.12 11.42	-0.12 11.17	-0.12 11.40	-0.12 11.57	-0.12 11.01	-0.12 11.24	-0.12 11.56	-0.12 11.62	-0.12 11.65	-0.12 11.56	-0.12 11.22	-0.12 10.77	-0.12 11.65	-0.12 11.56	-0.12 10.77	-0.12 10.77	-0.12 10.77	-0.12 10.77	-0.12 10.97	-0.12 11.59	-0.12 11.37	-0.12 10.27	-0.12 11.21	-0.12 11.60	-0.12 10.85	-0.12 10.75	-0.12 10.75	-0.12 11.25	-0.12 12.99	-0.12 10.85	-0.12 11.61	-0.12 10.68
26	Ser 29	0.13 74.74	0.15 73.64	0.13 73.34	0.19 71.14	0.15 73.32	0.14 74.44	0.14 73.04	0.14 72.98	0.13 74.43	0.14 73.94	0.14 68.79	0.14 69.42	0.15 72.13	0.14 73.11	0.14 71.81	0.15 72.75	0.15 72.09	0.14 72.75	0.14 72.61	0.15 72.71	0.14 73.46	0.15 71.61	0.14 71.30	0.14 73.31	0.14 73.31	0.14 73.31	0.13 72.86	0.14 71.27	0.14 73.17	0.13 70.99	0.15 75.80	0.15 71.30	0.15 73.06	0.15 70.54	0.14 72.08	0.15 72.25	0.20 68.09	0.19 70.51	0.15 71.94	0.15 74.40
27	Ala 30	0.44 25.26	0.42 25.26	0.42 25.26	0.39 25.26	0.42 25.26	0.44 25.26	0.42 25.26	0.36 25.26	0.44 25.26	0.42 25.26	0.34 25.26	0.35 25.26	0.46 25.26	0.41 25.26	0.45 25.26	0.45 25.26	0.40 25.26	0.45 25.26	0.45 25.26	0.41 25.26	0.45 25.26	0.38 25.26	0.41 25.26	0.45 25.26	0.41 25.26	0.45 25.26	0.41 25.26	0.45 25.26	0.39 25.26	0.39 25.26	0.45 25.26	0.38 25.26	0.42 25.26	0.39 25.26	0.45 25.26	0.39 25.26	0.36 25.26	0.42 25.26	0.40 25.26	0.45 25.26
28	Gly 29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
29	Asp 30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	Ile 31	9.04	9.71	7.86	8.37	8.87	8.54	8.03	8.20	8.87	8.87	8.87	8.70	7.69	7.86	8.03	9.21	8.87	9.21	9.02	8.54	9.04	8.87	8.87	8.87	8.87	8.87	8.37	8.87	8.03	8.37	10.05	7.53	7.69	8.20	8.87	7.53	8.87	8.03	8.03	8.87
31	Met 32	0.14 21.76	0.16 22.26	0.13 22.60	0.17 25.61	0.14 22.26	0.14 22.26	0.13 21.43	0.13 22.43	0.13 23.60	0.14 18.75	0.14 22.43	0.14 24.11	0.14 22.59	0.14 22.59	0.13 21.76	0.14 21.76	0.14 22.76	0.15 22.93	0.14 22.25	0.14 20.44	0.14 23.09	0.14 23.10	0.14 21.76	0.14 22.10	0.14 22.10	0.14 22.10	0.14 23.94	0.14 22.59	0.14 21.42	0.14 24.78	0.15 22.59	0.14 21.75	0.14 24.94	0.14 24.59	0.14 25.28	0.14 26.43	0.14 22.42	0.14 21.25	0.14 24.11	0.14 22.02
32	Ile 54	0.00 0.44	0.29	0.00 0.29	0.00 0.15	0.00 0.29	0.00 0.29	0.00 0.44	0.00 0.15	0.00 0.44	0.00 0.29	0.00 0.44	0.00 0.44	0.00 0.44	0.00 0.44	0.00 0.58	0.00 0.29	0.00 0.44	0.00 0.15	0.00 0.44	0.00 0.58	0.00 0.44	0.00 0.29	0.00 0.44	0.00 0.44	0.00 0.44	0.00 0.44	0.00 0.44	0.00 0.58	0.00 0.29	0.00 0.44	0.00 0.44	0.00 0.15	0.00 0.44	0.00 0.44	0.00 0.44	0.00 0.29	0.00 0.44	0.00 0.44	0.00 0.29	0.00 0.58
33	Thr 33	-0.00 55.22	-0.00 57.75	-0.00 54.67	-0.00 42.61	-0.00 60.97	-0.00 59.91	-0.00 56.87	-0.00 58.60	-0.00 47.63	-0.00 55.20	-0.00 57.09	-0.00 48.62	-0.00 47.89	-0.00 57.23	-0.00 47.01	-0.00 60.10	-0.00 58.78	-0.00 59.08	-0.00 60.00	-0.00 46.42	-0.00 47.13	-0.00 59.36	-0.00 58.45	-0.00 48.97	-0.00 48.97	-0.00 48.97	-0.00 49.97	-0.00 54.11	-0.00 49.87	-0.00 50.29	-0.00 58.59	-0.00 48.61	-0.00 46.57	-0.00 51.83	-0.00 41.83	-0.00 45.14	-0.00 60.97	-0.00 48.62	-0.00 43.70	-0.00 58.66
34	Val 34	-0.47 63.35	-0.28 66.28	-0.40 63.01	-0.29 62.54	-0.25 66.88	-0.30 66.33	-0.48 71.61	-0.29 64.57	-0.31 67.26	-0.38 67.37	-0.40 66.88	-0.28 65.90	-0.25 64.25	-0.38 66.87	-0.36 68.22	-0.30 66.50	-0.39 66.51	-0.24 64.82	-0.35 65.95	-0.32 63.62	-0.35 63.64	-0.31 64.99	-0.34 67.31	-0.41 69.42	-0.41 69.42	-0.41 69.42	-0.41 69.42	-0.37 64.33	-0.37 66.90	-0.28 65.05	-0.38 63.57	-0.36 63.94	-0.36 69.59	-0.22 63.52	-0.30 69.81	-0.35 66.69	-0.34 72.31	-0.36 65.92	-0.36 68.95	-0.35 66.72	-0.35 69.43
35	Leu 36	-1.37	-1.28	-1.40	-1.29	-1.25	-1.35	-1.48	-1.29	-1.31	-1.38	-1.45	-1.28	-1.25	-1.38	-1.36	-1.30	-1.39	-1.24	-1.35	-1.32	-1.35	-1.31	-1.34	-1.41	-1.41	-1.41	-1.41	-1.37	-1.37	-1.28	-1.38	-1.36	-1.22	-1.30	-1.35	-1.34	-1.36	-1.31	-1.35	-1.35
36	Ala 74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Supplementary table S5. Interface analysis of CAS SH3-Vinculin (SH3 part).
Hydrogen bonds and nonpolar contacts between Cas SH3 and its ligands were analyzed with PISA with default settings. Prior to analysis, the linker sequence (residues 75-82) was removed from the chimeric proteins. Solvent accessible residues are colored yellow and inaccessible residues are blue. ΔG represents free energy of dissociation (solvation energy effect, kcal/mol). BSA means Buried Surface Area (Å²) and is displayed with bars, one bar per 10%. HSDC indicates residues that contain the across-interface hydrogen bond, salt bridge, or disulfide bond atoms. The corresponding table cells have a brown background and contain letter H for a hydrogen bond, S for a salt bridge, and HS for a combination of hydrogen bond and salt bridge.
http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html



Supplementary table S6. Interface analysis of CAS SH3-Vinculin (ligand part).
Hydrogen bonds and nonpolar contacts between Cas SH3 and its ligands were analyzed with PISA with default settings. Prior to analysis, the linker sequence (residues 75-82) was removed from the chimeric proteins. Solvent accessible residues are colored yellow and inaccessible residues are blue. ΔiG represents free energy of dissociation (solvation energy effect, kcal/mol). BSA means Buried Surface Area (Å^2) and is displayed with bars, one bar per 10%. HSDC indicates residues that contain the across-interface hydrogen bond, salt bridge, or disulfide bond atoms. The corresponding table cells have a brown background and contain letter H for a hydrogen bond, S for a salt bridge, and HS for a combination of hydrogen bond and salt bridge.
http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html

Hydrogen bonds (interface) XML

##	UNIPROT	CAS_SH3	Vinculin		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	Arg25	A:ARG 27[NH1]	B:LEU 83[O]	-6																																					X			
2	Lys26	A:LYS 28[NZ]	B:THR 84[OG1]	-5																	X																							
3	Arg25	A:ARG 27[NH1]	B:THR 84[OG1]	-5																																					X	X		
4		A:ARG 27[NH2]	B:THR 84[OG1]	-5																																						X		
5	Lys26	A:LYS 28[NZ]	B:THR 84[O]	-5																													X								X			
6	Lys26	A:LYS 28[NZ]	B:ASP 85[O]	-4																			X										X								X			
7	Lys69	A:LYS 71[NZ]	B:GLU 86[OE2]	-3																					X																			
8	Lys26	A:LYS 28[NZ]	B:GLU 86[O]	-3																			X																					
9	Tyr12	A:TYR 14[HH]	B:GLU 86[OE2]	-3																					X		X						X											
10	Lys26	A:LYS 28[NZ]	B:GLU 86[OE2]	-3		X	X					X		X	X	X	X									X	X				X	X		X	X			X	X		X		X	
11		A:LYS 28[NZ]	B:GLU 86[OE1]	-3				X	X	X	X		X	X	X			X				X	X		X	X					X				X	X			X					
12	Arg59	A:ARG 61[HE]	B:PRO 89[O]	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
13	Arg59	A:ARG 61[NH2]	B:PRO 89[O]	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
14	Asn58	A:ASN 60[ND2]	B:PRO 90[O]	1	X	X	X	X	X	X	X	X		X	X			X		X	X	X	X			X	X				X		X	X	X						X			X
15	Val15	A:VAL 17[O]	B:LYS 91[NZ]	2	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
16	Glu21	A:GLU 23[OE1]	B:LYS 91[NZ]	2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
17		A:GLU 23[OE2]	B:LYS 91[NZ]	2	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
18	Glu17	A:GLU 19[OE1]	B:LYS 91[NZ]	2								X	X				X			X	X		X	X		X	X	X	X	X	X		X	X		X				X	X	X		X
19		A:GLU 19[OE2]	B:LYS 91[NZ]	2	X	X	X	X	X	X	X				X	X		X	X			X			X							X			X		X	X	X				X	

Salt bridges (Interface) XML

##	UNIPROT	CAS_SH3	Vinculin		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	Lys26	A:LYS 28[NZ]	B:GLU 86[OE1]	-3	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X		X	X				X
2		A:LYS 28[NZ]	B:GLU 86[OE2]	-3	X	X	X		X			X		X	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X	X
3	Glu21	A:GLU 23[OE1]	B:LYS 91[NZ]	2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4		A:GLU 23[OE2]	B:LYS 91[NZ]	2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
5	Glu17	A:GLU 19[OE1]	B:LYS 91[NZ]	2					X			X	X	X			X			X	X		X	X		X	X	X	X	X	X		X	X		X				X	X	X		X
6		A:GLU 19[OE2]	B:LYS 91[NZ]	2	X	X	X	X		X	X			X	X	X		X	X			X			X		X					X			X		X	X	X		X		X	

SDE	Pocket S1	Pocket S2	Pocket S3	SDE (ext. S3)
Arg25				
Lys26				
Arg25				
Lys26				
Lys26				
Lys69				
Lys26				
Tyr12	Tyr12			
Lys26				
	Arg59	Arg59		
	Arg59	Arg59		
	Asn58	Asn58		
		Val15		
		Glu21	Glu21	
		Glu17	Glu17	
Lys26				
		Glu21	Glu21	
		Glu17	Glu17	

SH3 numbering from UNIPROT: X-2

number of structures
0-5 %
6-20 %
21-50 %
51-80%
81-100%

	Vinculin			UNIPROT
	-6	L	83	854
	-5	T	84	855
SDE	-4	D	85	856
	-3	E	86	857
	-2	L	87	858
	-1	**A**	88	859
	0	**P**	89	860
	1	P	90	861
core	2	**K**	91	862
	3	**P**	92	863
	4	P	93	864
	5	**L**	94	865
	6	P	95	866
	7	E	96	867
SDE	8	G	97	868
	9	E	98	869
	10	V	99	870

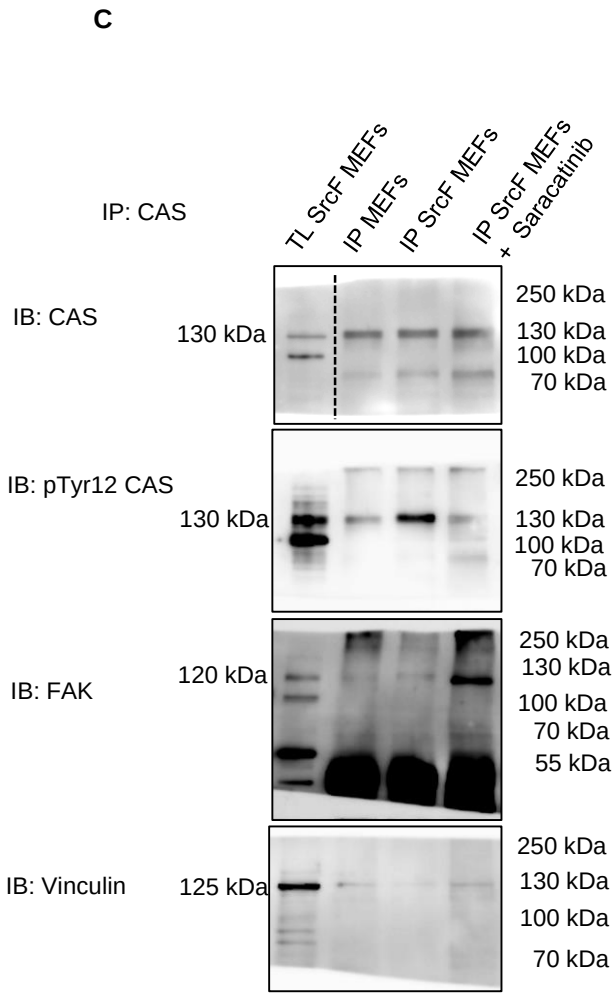
Supplementary table S7. Interface analysis of CAS SH3-Vinculin (hydrogen bonds).
Hydrogen bonds and salt bridges between Cas SH3 and its ligands were analyzed with PISA with default settings. Prior to analysis, the linker sequence (residues 75-82) was removed from the chimeric proteins. The atoms that form hydrogen bonds and salt bridges are indicated by crosses.

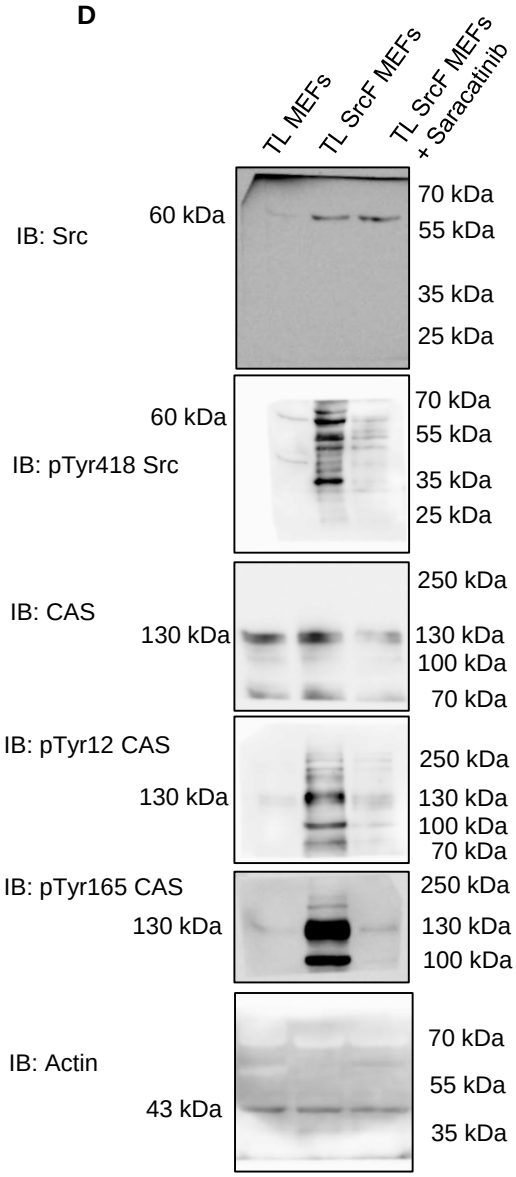
CAS_SH3-ligand_variant	Int.ene	Disp	H-bond
CAS-PTP_PEST-A12	-115.3	-55.8	-24.5
CAS-PTP_PEST-E12	-114.5	-58.3	-25.2
CAS-PTP_PEST-F12	-110.7	-60.7	-24.0
CAS-PTP_PEST-Y12	-124.6	-61.9	-30.8
CAS-PTP_PEST-pY12	-103.2	-57.8	-25.9

CAS_SH3-ligand_variant	Int.ene	Disp	H-bond
CAS-Vinculin-A12	-89.4	-56.5	-19.0
CAS-Vinculin-E12	-93.1	-61.8	-19.6
CAS-Vinculin-F12	-102.8	-63.5	-19.0
CAS-Vinculin-Y12	-104.1	-63.5	-19.1
CAS-Vinculin-pY12	-83.7	-57.5	-18.5

Supplementary table S8. Interaction energies – Quantum mechanics.
Interaction energies (Int. ene) and their dispersion (Disp) and hydrogen-bonding (H-bond) components of CAS vs. PTP-PEST/Vinculin binding for the wild-type, Tyr12 phosphorylated, and Tyr12 mutated CAS variants. All values are in kcal/mol.

Uncropped immunoblots for Figure 7:





Uncropped immunoblots for Figure 8: