

Supporting Information

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**The RING Domain of the Scaffold Protein Ste5 Adopts a Molten Globular Character with High Thermal and Chemical Stability\*\***

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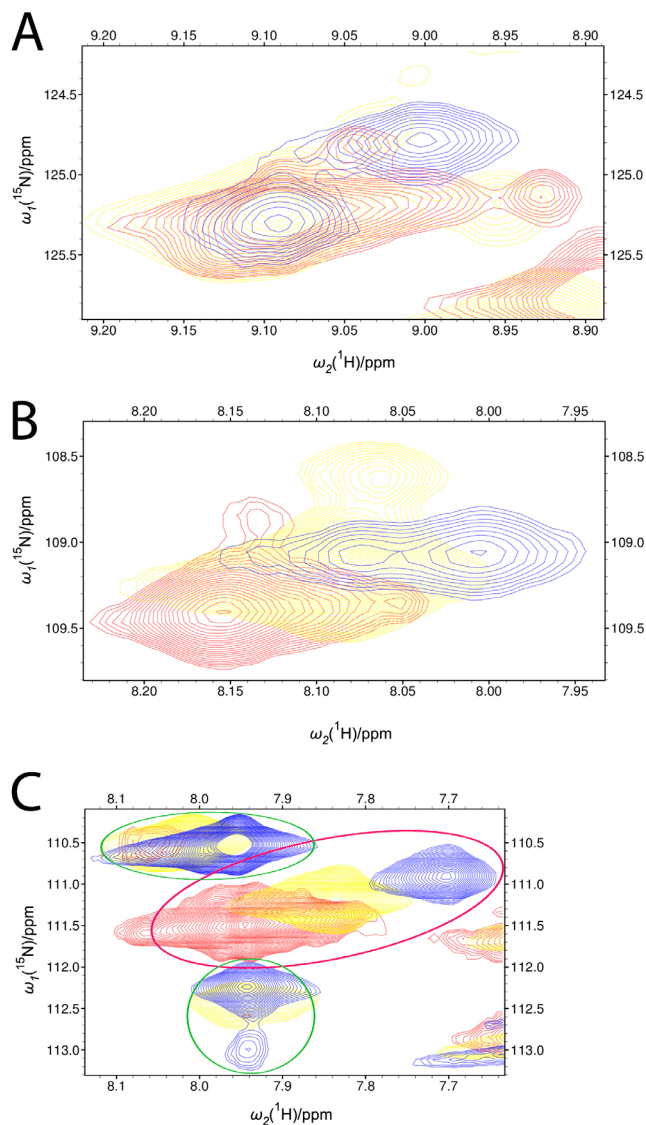
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# Supporting Information

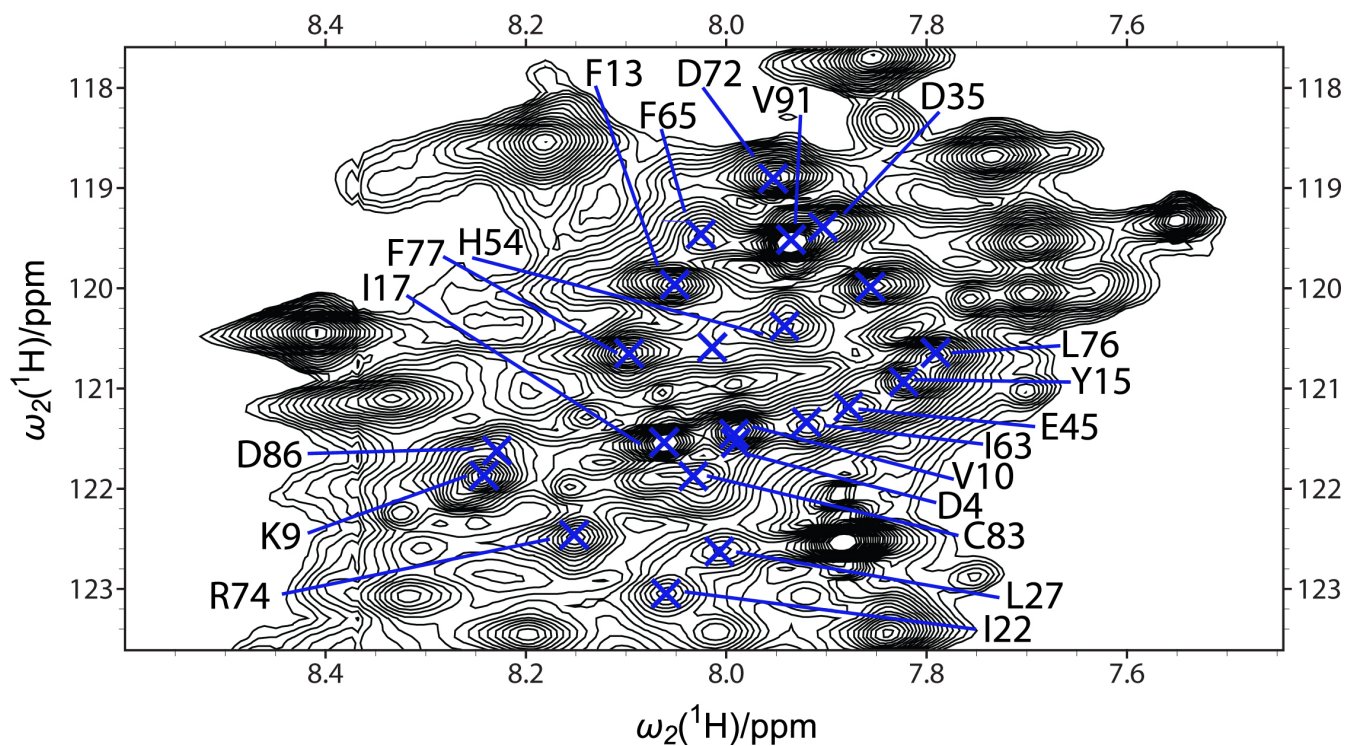
## **Preparation of yeast extract**

Yeast cells were grown in SGal media at 30 °C for 24 hours. Afterwards, the cells were crushed in a freezer mill and centrifuged at 17000 rpm. 8 grams of the obtained pellet were then re-suspended in 10 mM Tris-HCl pH 7.5, 150 mM KCl, 2 mM MgCl<sub>2</sub>, 0.1% NP-40. A single tablet of Roche complete EDTA free protease inhibitor was added as well as 100 µM PMSF. This mixture was loaded onto a Chelex-100 resin. After 5 minutes incubation the resin was centrifuged at 17000 rpm and the supernatant retained. The supernatant was further centrifuged at 190000 ref for 60 minutes at 2 °C. The supernatant was flash frozen in liquid nitrogen and stored at a temperature of -80 °C.

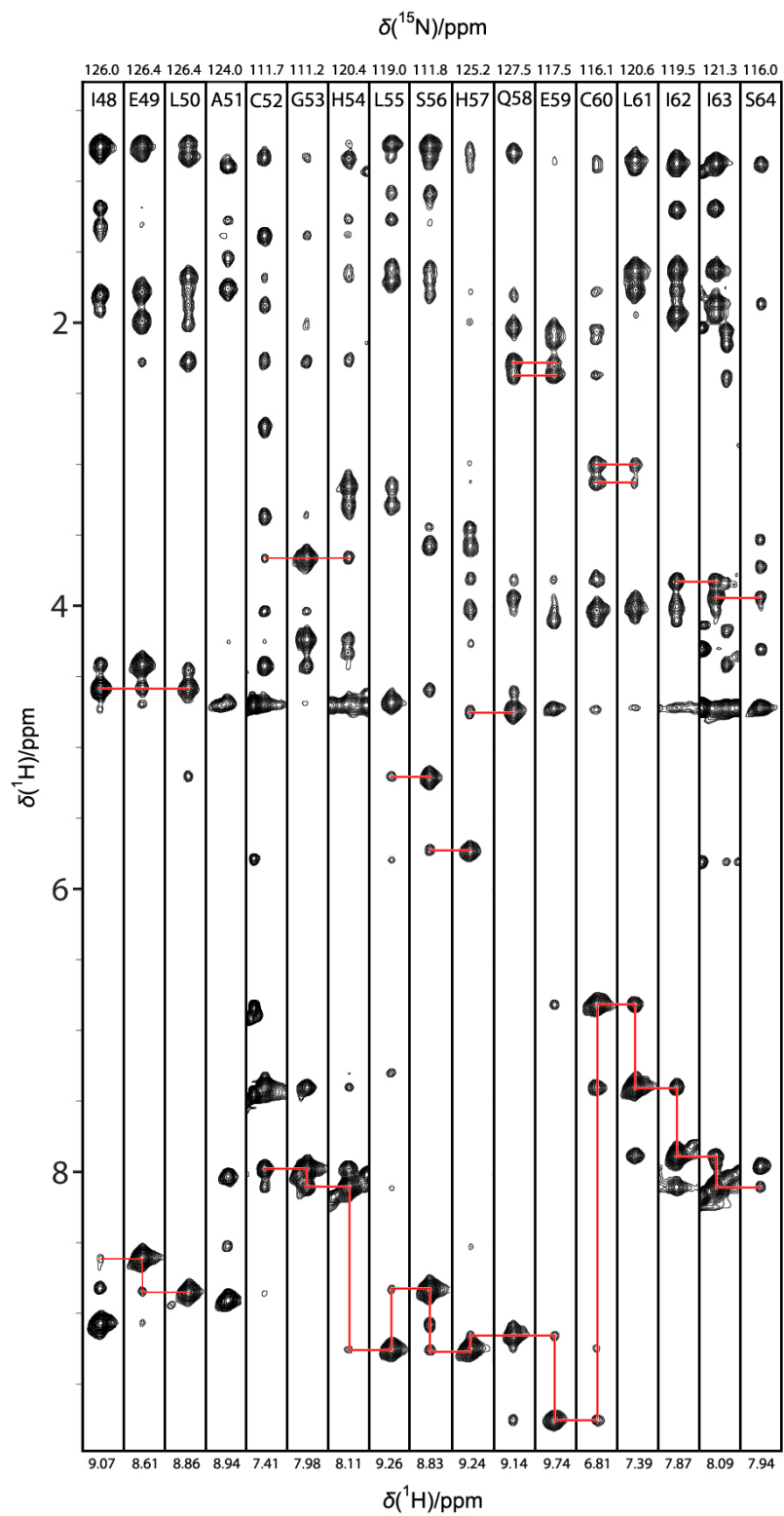
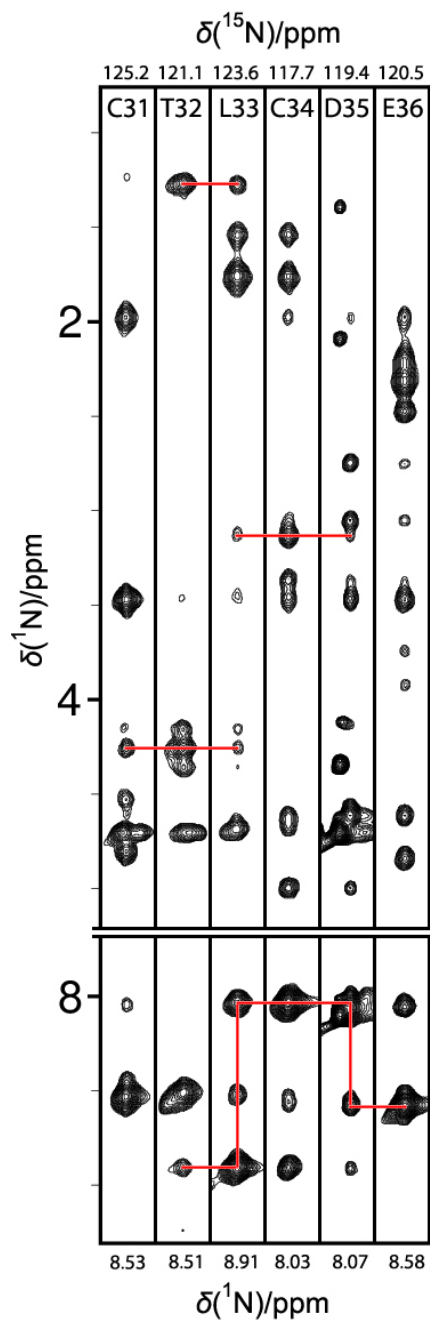
## Supplementary Figures

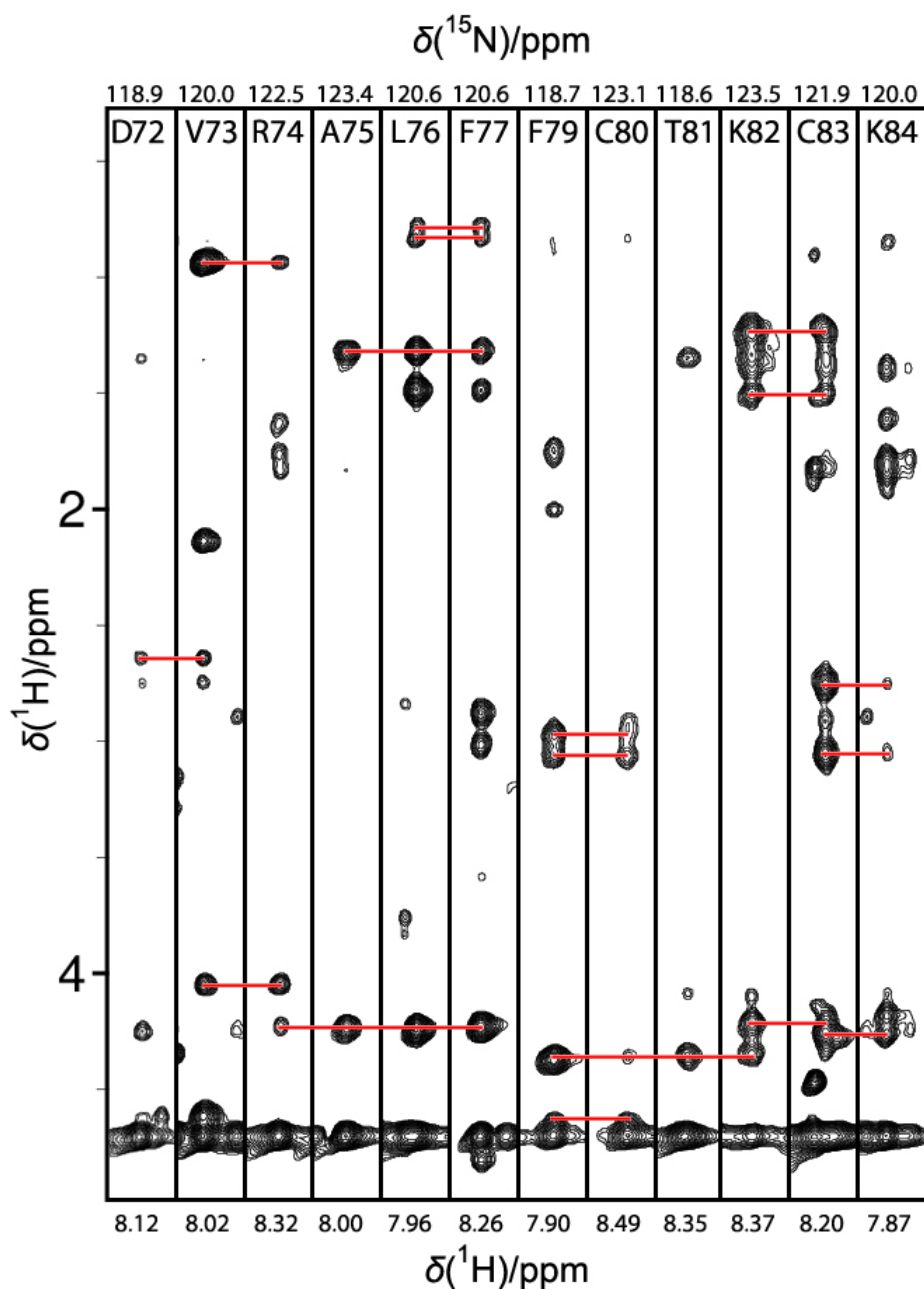


**Figure S1:** Overlays of expansions of  $[^1\text{H}, ^{15}\text{N}]$  – HSQC spectra of scSte5 RH2 (*S. cerevisiae* Ste5 RING-H2 domain) measured at three different temperatures (Fig. 3): 5 (blue), 25 (yellow) and 45 °C (red) at 900 MHz. The spectra were calibrated to compensate for temperature shifts and show peak doubling. A) peak doubling with approximately equal distribution of conformers at 5 °C (blue peaks) turns into peak tripling at 25 and 45 °C with dominant population of one conformer over two others. B) at 4 °C peak doubling indicating two conformers, at 25 °C peak tripling (in  $^{15}\text{N}$  dimension), and at 45 °C almost coalescence. C) magenta ellipse indicates a resonance that demonstrates slight peak doubling only at 45 °C with significant line-width narrowing towards lower temperatures; green ellipses show resonances that disappear at 45 °C probably due to line broadening.

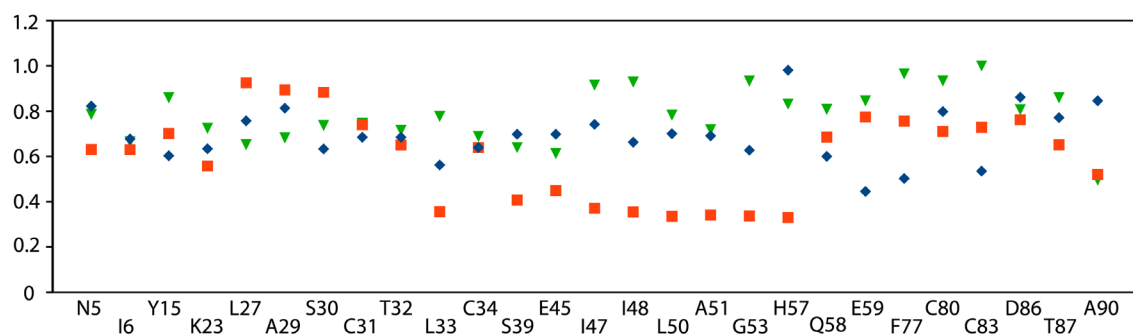


**Figure S2:** Central fragment of 2D  $^1\text{H} - ^{15}\text{N}$  correlation spectrum of scSte5 RH2 measured at 900 MHz at 25 °C (expansion of Figure 4A); backbone resonance assignments are indicated using the one letter amino acid code.



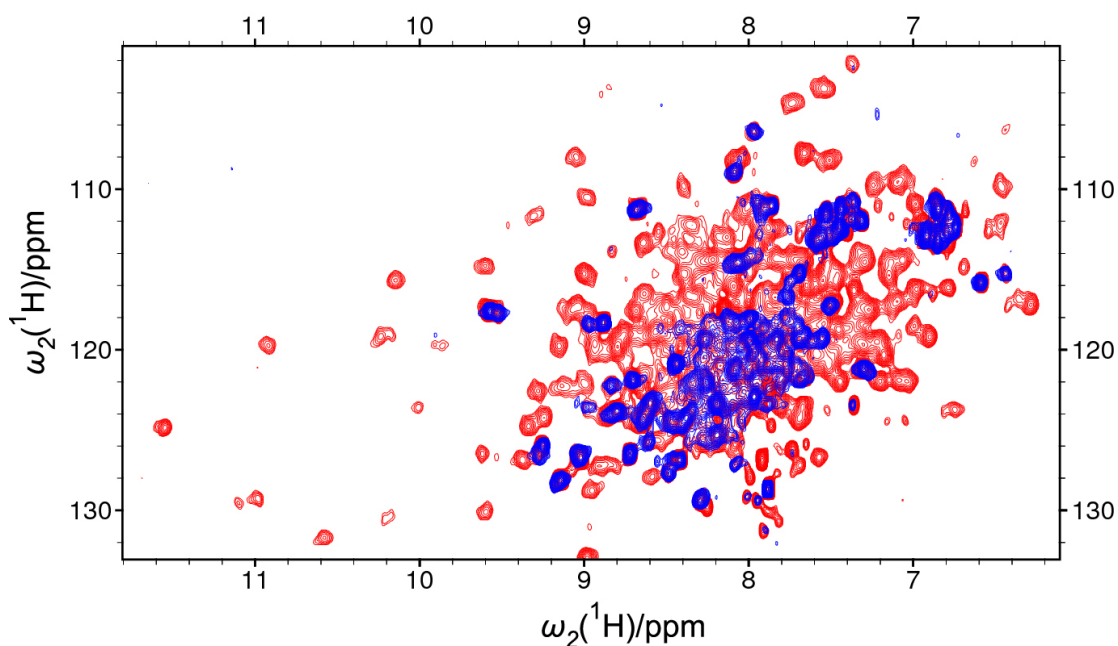


**Figure S3:** Strips from a 3D  $^{15}\text{N}$  resolved  $[^1\text{H},^1\text{H}]$ -NOESY spectrum taken along the indirect  $^1\text{H}$  dimension centered at the amide proton chemical shifts of the residues indicated at the top of the strips, where also the  $^{15}\text{N}$  chemical shift is given (peak list given in Table S5). The strips document the existence of helical secondary structure in *S. cerevisiae* RING-H2 domain (scSte5 RH2). The red lines connect sequential NOEs typical for  $\alpha$ -helical segments.

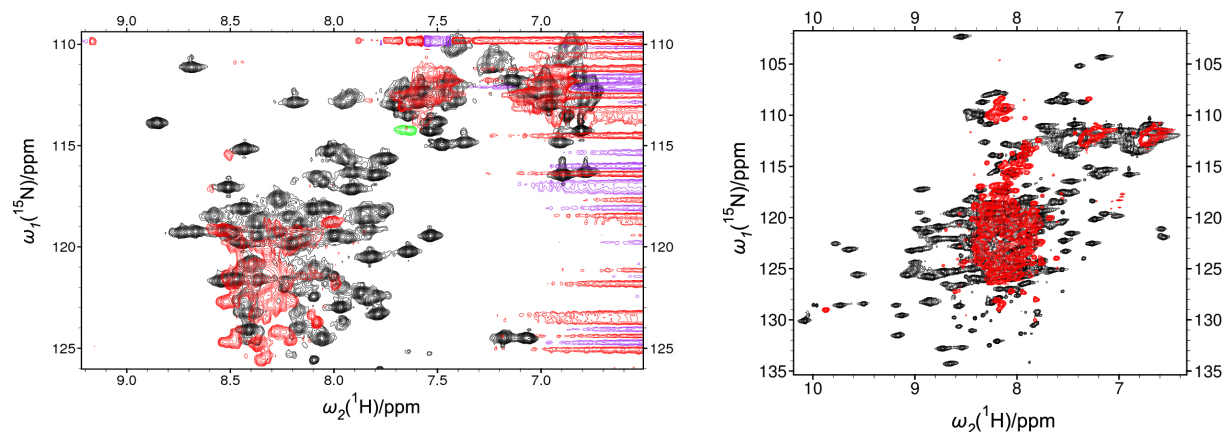


**Figure S4:**

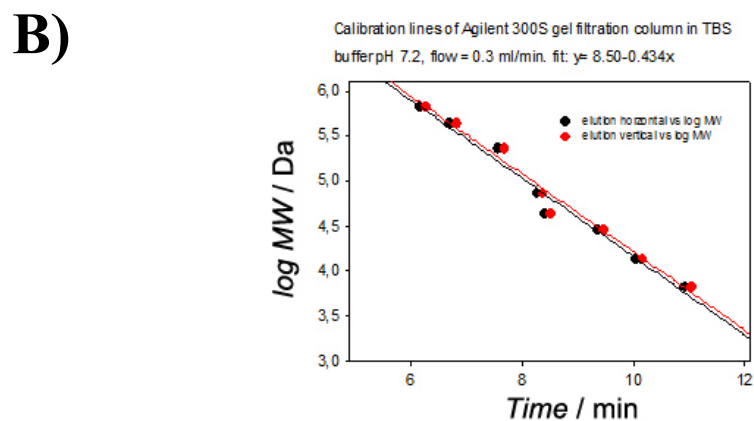
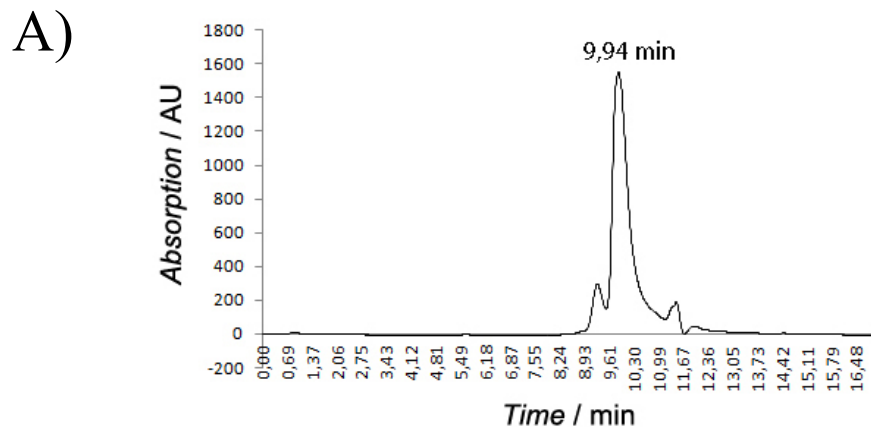
$^{15}\text{N}\{^1\text{H}\}$  heteronuclear NOE data of the Ste5 RING-H2 domain versus the amino acid sequence showing only the assigned, well resolved residues. The NOE data was measured in three different buffer conditions: standard buffer as described in Figure 1 (blue rhomboids), standard buffer containing 7 M urea (red squares), and in yeast extracts (green triangles). All measurements were performed at 500 MHz at a temperature of 25 °C and at a concentration of 500  $\mu\text{M}$  scSte5 RH2.



**Figure S5:** Overlay of two  $^1\text{H} - ^{15}\text{N}$  correlation spectra of  $^{15}\text{N}$  labeled scSte5 RING-H2 measured in the buffer described in Figure 1 (blue) and in complex with G protein  $\beta/\gamma$  subunits (Ste4/Ste18) (red). The spectra were measured at 600 MHz at a temperature of 25 °C; the concentrations of scSte5 RH2 and of Ste4/Ste18 were 10  $\mu\text{M}$  and 20  $\mu\text{M}$ , respectively.

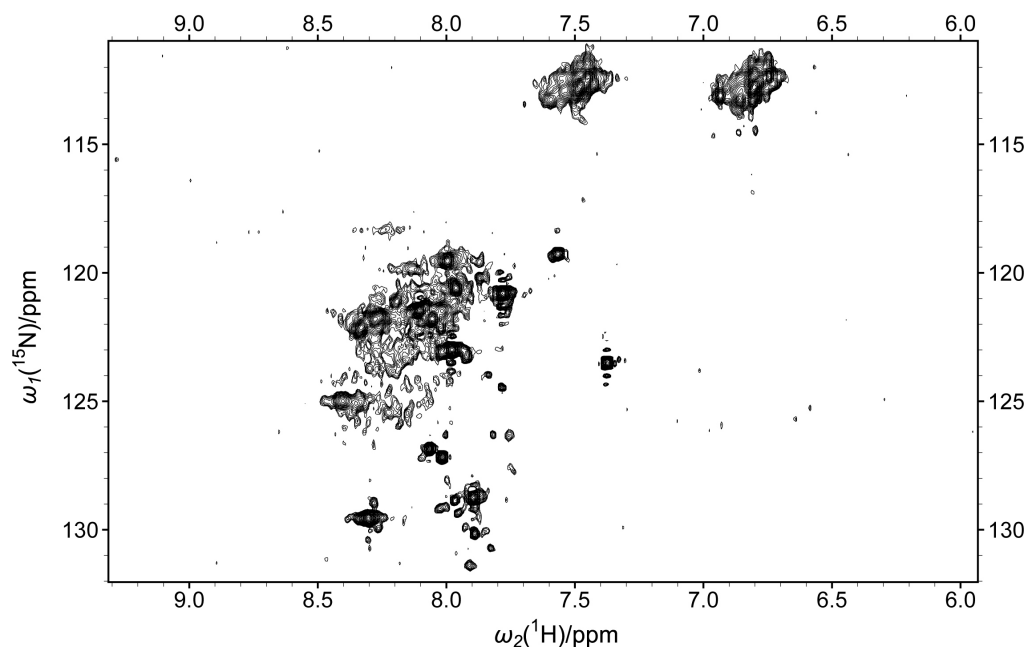


**Figure S6:** Effect of 7M urea on other zinc-finger proteins: yeast Far1 RING domain (left) and MBNL-1 (muscle blind-like protein from higher eukaryotes) zinc finger protein (right). Shown are overlays of  $[^1\text{H}, ^{15}\text{N}]$  – HSQC spectra measured in standard non-denaturing buffer (black) and in the same buffer containing in addition 7 M urea (red). The overall fold disruption upon addition of urea is clearly visible for both proteins. The left set of spectra shows baseline distortions due to the intense resonance of urea.

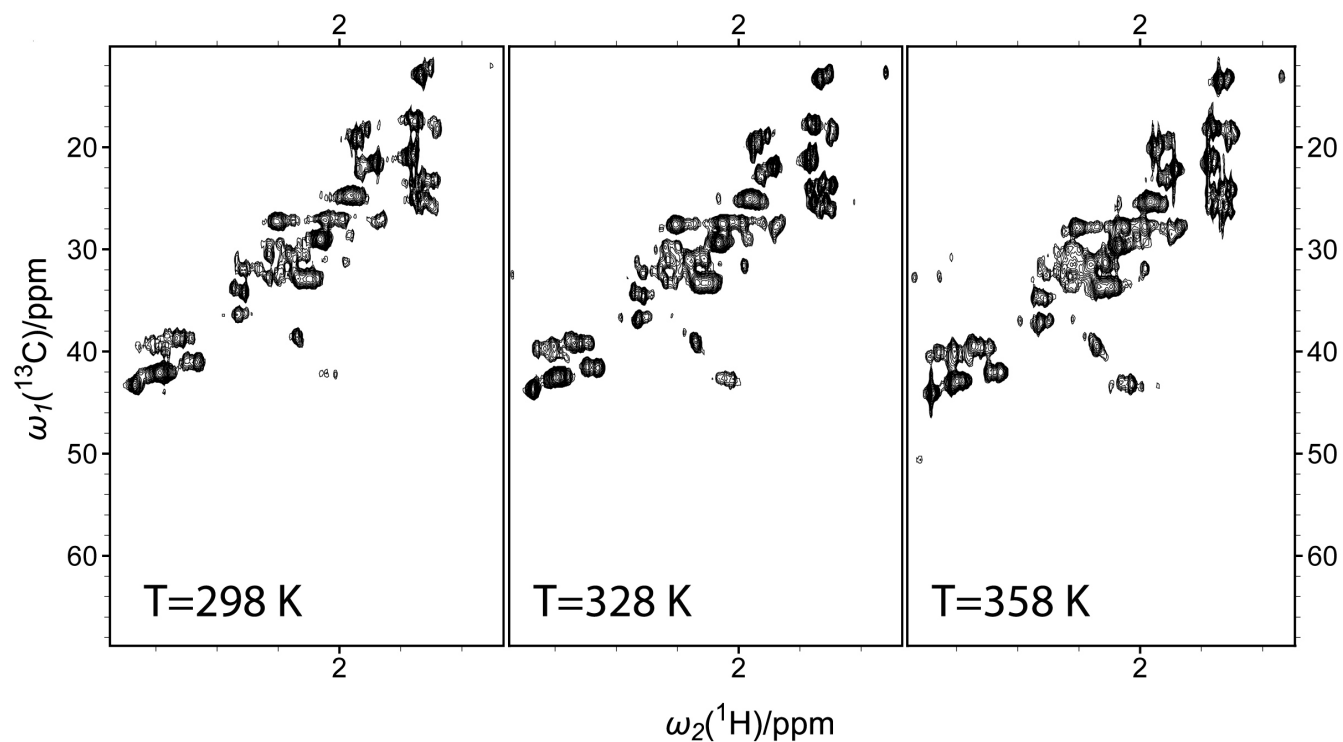




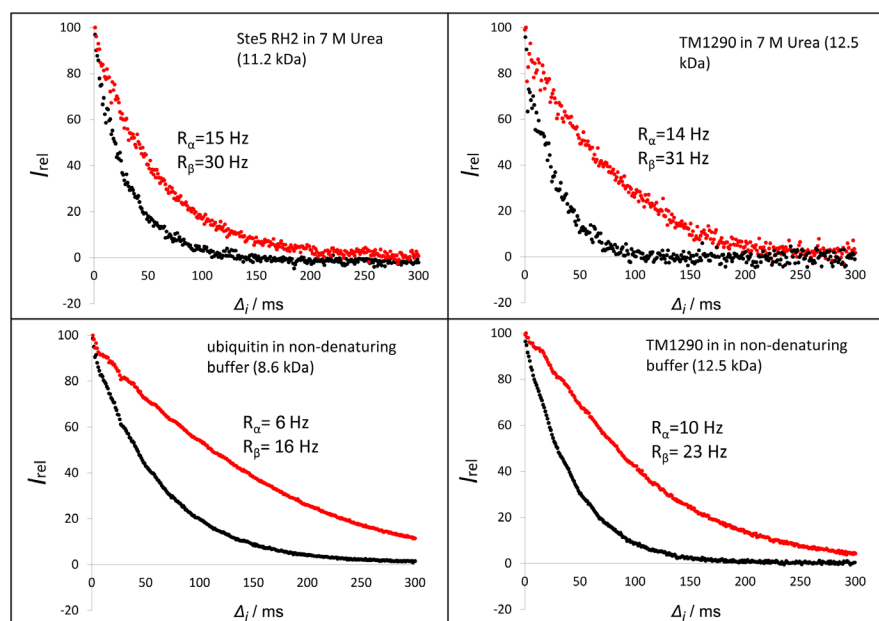
**Figure S7:** A) analytical gel filtration curve performed with Agilent 300S column at horizontal orientation in standard buffer conditions at 200  $\mu\text{M}$  (Figure 1). The RING-H2 domain elutes at 9.94 minutes which corresponds to molecular weight of 15.1 kDa; the sharp monodisperse peak of the Ste5 RING-H2 domain shows no aggregation signatures. Two small peaks at larger and smaller elution volumes are impurities which are always excluded from further experiments. The molecular weight based on the retention time (15.1 kDa) is higher than expected for scSte5 RH2 (11.3 kDa) which might be due to larger hydrodynamic radius of the MG Ste5 RH2 domain or slightly different conditions of the column calibration. B) calibration curve of the Agilent 300S column at pH = 7.2 in TBS buffer as recommended by the manufacturer (linear regression fit:  $y=8.5-0.434x$ ); red points correspond to a vertical, black points to a horizontal orientation of the column.



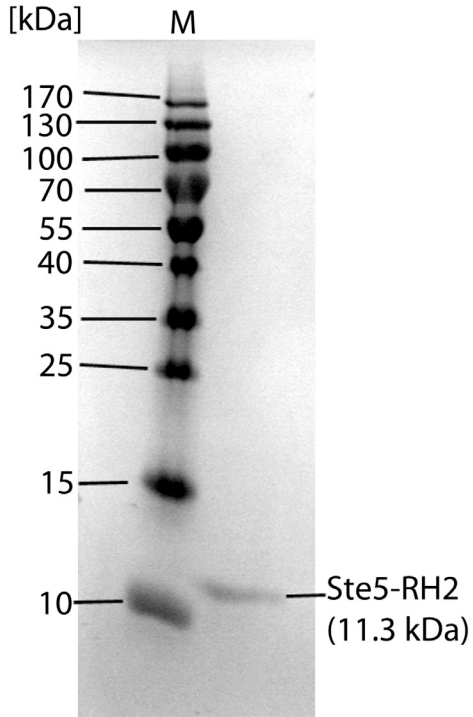
**Figure S8:** [ $^1\text{H}$ ,  $^{15}\text{N}$ ] – HSQC spectrum of the 200  $\mu\text{M}$  Ste5 RING-H2 domain in standard buffer conditions, at 25  $^\circ\text{C}$  after addition of EDTA (1 mM). This spectrum demonstrates that the Ste5 MG state has zinc ions bound; compare with Fig. 2 or Fig. S5: the dispersed resonances of the zinc coordinating residues have disappeared. Further evidence that the MG state binds zinc comes from the the beta carbons of cysteine residues which coordinate zinc have typical chemical shift values for zinc coordination <sup>[1]</sup>. The corresponding  $^{13}\text{C}$  chemical shift values are listed in Table S3 in the Supporting Information.



**Figure S9:** Methyl region of [ $^1\text{H}$ ,  $^{13}\text{C}$ ] – HSQC spectra of the 200  $\mu\text{M}$  Ste5 RING-H2 domain in standard buffer conditions at temperatures 298 K, 328 K and 358 K from left to right, respectively. There are no significant temperature dependent chemical shift changes indicating that no thermal unfolding is taking place.



**Figure S10:** Results from TRACT <sup>[2]</sup> relaxation measurements to confirm the monomeric state of scSte5 H2. TRACT allows an estimate of the size of a macromolecule in solution without interference from chemical exchange or dipole–dipole coupling of the amide proton with remote protons. TRACT makes use of cross-correlation between chemical shift anisotropy (CSA) and dipole-dipole (DD) relaxation in the amide groups and measures the relaxation rates,  $R_\alpha$  and  $R_\beta$ , of the two transitions of a <sup>15</sup>N nucleus (red and black curves in the figure). Half the difference of  $R_\alpha$  and  $R_\beta$  is equal to the transverse cross-correlated relaxation rate,  $\eta_{xy}$ . With increasing molecular weight  $\eta_{xy}$  becomes larger. The calculation of e.g. rotational correlation times (representing molecular weights) from relaxation data requires a model of motion and the shape of the molecule. For compact, folded proteins often an isotropically tumbling sphere is assumed. For molten globular states such a simplification may not be justified. For this reason we compare  $\eta_{xy}$  of scSte5 RH2 with TM1290 which has similar size. TM1290 is a well folded, very stable, monomeric 12.5 kDa protein from *Thermatoga maritima* <sup>[3]</sup>. We measured the relaxation rates  $R_\alpha$  and  $R_\beta$  of scSte5 RH2 and TM1290 both in 7M urea, as well as for TM1290 and ubiquitin in non-denaturing conditions. All measurements were performed at 750 MHz at 298 K. The sample conditions were: 1.5 mM scSte5 RH2 in 7 M Urea, pH 7.2; 1.5 mM TM1290 in 20 mM sodium phosphate; 1.5 mM TM1290 in 20 mM sodium phosphate and 7 M urea, pH 6.2; 2.5 mM ubiquitin in 20 mM sodium phosphate, pH 6.0. The measured relaxation rates  $R_\alpha/R_\beta$  and the resultant rotational correlation times  $\tau_c$  (given in brackets) were for scSte5 RH2 in 7M urea, TM1290 in 7M urea, ubiquitin and TM1290, 15/30 Hz (6.0 ns), 14/31 Hz (6.6 ns), 6/16 Hz (3.8 ns), and 10/23 Hz (5.2 ns), respectively; repetitive fitting with different number of data points indicates an error of +/-15% for the samples in 7 M urea and an error of +/- 10% for the non-denaturing conditions. Due to the higher viscosity and probably increased hydrodynamic radius proteins tumble more slowly in 7M urea as can be seen for TM1290 which does not unfold in 7 M urea. The results from the TRACT measurements show that scSte5 RH2 is monomeric under the given solution conditions since scSte5 RH2 and TM1290 both in urea give very similar results. Ubiquitin was measured as a control and is known to have a  $\tau_c$  of about 4 ns in the given conditions.



**Figure S11:** SDS-PAGE gel demonstrating that zinc ions are coordinated within one domain of scSte5 RH2; first lane, M, presents a molecular weight marker, the second lane scSte5 RH2.



**Figure S12:** Amino acid sequence of the studied RING-H2 domain of scSte5 using the one-letter code for amino acids. Large capital letters indicate residues which are assigned in the NMR spectrum, small letters stand for residues which are not assigned. In red are shown residues for which doubling and/or tripling at given temperature is well observed, in green residues for which peaks are not visible at 45 °C, and asterisks indicate residues for which resonances disappear at 5 °C in [<sup>1</sup>H, <sup>15</sup>N] – HSQC spectra (Figs. 3 and S1). The first two residues, in grey, derive from the expression vector.

## Supplementary Tables

**Table S1:** NMR experiments used for backbone assignment.

Experiment
[ <sup>1</sup> H, <sup>15</sup> N] - HSQC
HNCA <sup>[4]</sup>
HN(CO)CA <sup>[5]</sup>
HNCO <sup>[6]</sup>
HN(CA)CO <sup>[7]</sup>
HNCACB <sup>[8]</sup>
CBCA(CO)NH <sup>[9]</sup>
<sup>15</sup> N NOESY <sup>[10]</sup>
4D APSY-HNCACB <sup>[11]</sup>
5D APSY-CBCACONH <sup>[12]</sup>
4D APSY-CBCANH <sup>[12]</sup>

**Table S2:** Secondary structure prediction with program TALOS+ <sup>[13]</sup>.

VARS	RESID	RESNAME	PHI	PSI	DPHI	DPSI	DIST	S2	COUNT	CS_COUNT	CLASS	
FORMAT	%4d	%s	%8.3f	%8.3f	%8.3f	%8.3f	%8.3f	%5.3f	%2d	%2d	%s	
3	S	9999.000	9999.000		0.000	0.000	0.000	0.000	0.000	0	12	None
4	D	-76.910	-25.889	18.951	19.243	45.497	0.638	10	10	Warn		
5	N	-65.785	136.984	57.483	22.147	34.007	0.711	8	12	Warn		
6	I	-101.893	143.903	27.074	22.871	74.462	0.771	10	8	Warn		
7	?	9999.000	9999.000		0.000	0.000	0.000	0	6	None		
8	P	9999.000	9999.000		0.000	0.000	0.000	0	6	None		
9	K	-83.304	134.222	74.098	42.266	62.321	0.778	10	9	Warn		
10	V	-104.506	130.901	32.821	12.034	78.721	0.700	10	7	Warn		
11	?	9999.000	9999.000		0.000	0.000	0.000	0	5	None		
12	P	9999.000	9999.000		0.000	0.000	0.000	0	6	None		
13	F	-64.739	131.502	79.799	46.245	78.290	0.749	7	9	Warn		
14	G	86.360	1.641	10.322	14.167	89.362	0.603	10	11	Warn		
15	Y	-93.591	134.655	18.159	24.716	85.039	0.480	10	9	Dyn		
16	P	-65.475	153.618	8.758	8.483	58.631	0.461	10	10	Dyn		
17	I	-107.923	126.942	15.232	15.748	48.377	0.440	10	10	Dyn		
18	Q	-82.606	134.197	21.389	23.951	72.695	0.434	10	8	Dyn		
19	?	9999.000	9999.000		0.000	0.000	0.000	0	4	None		
20	?	9999.000	9999.000		0.000	0.000	0.000	0	0	None		
21	?	9999.000	9999.000		0.000	0.000	0.000	0	4	None		
22	I	-104.805	129.174	24.224	13.274	53.946	1.000	10	8	Warn		
23	K	-93.905	137.530	28.340	21.485	73.025	1.000	9	8	Warn		
24	?	9999.000	9999.000		0.000	0.000	0.000	0	4	None		
25	?	9999.000	9999.000		0.000	0.000	0.000	0	0	None		
26	?	9999.000	9999.000		0.000	0.000	0.000	0	4	None		
27	L	9999.000	9999.000		0.000	0.000	0.000	0	4	None		
28	?	9999.000	9999.000		0.000	0.000	0.000	0	4	None		
29	?	9999.000	9999.000		0.000	0.000	0.000	0	4	None		

30	S	-120.226	135.195	21.262	23.850	54.052	1.000	10	8	Warn
31	C	-124.865	135.986	17.717	14.713	36.575	0.907	10	12	Good
32	T	-75.886	135.540	12.947	18.309	44.740	0.816	10	12	Good
33	L	-93.397	-32.562	16.203	11.973	45.143	0.754	10	12	Good
34	C	-76.338	-27.526	23.970	22.846	43.564	0.757	8	12	Warn
35	D	-80.061	-19.376	6.225	15.774	52.942	0.762	10	12	Good
36	E	-109.882	117.738	28.867	38.026	82.535	0.702	10	10	Warn
37	P	-68.200	148.619	10.388	5.476	67.343	0.643	10	10	Good
38	I	-95.396	142.745	21.409	30.270	54.311	0.610	8	10	Warn
39	S	-122.316	136.323	44.152	23.461	74.391	0.611	8	8	Warn
40	?	9999.000	9999.000	0.000	0.000	0.000	0.000	0	4	None
41	?	9999.000	9999.000	0.000	0.000	0.000	0.000	0	0	None
42	?	9999.000	9999.000	0.000	0.000	0.000	0.000	0	0	None
43	?	9999.000	9999.000	0.000	0.000	0.000	0.000	0	1	None
44	G	9999.000	9999.000	0.000	0.000	0.000	0.000	0	5	None
45	E	-91.151	135.104	63.837	22.477	69.286	0.866	10	9	Warn
46	K	-104.842	137.586	67.156	20.930	37.339	0.861	10	12	Warn
47	I	-106.225	132.745	11.779	10.820	36.049	0.824	10	12	Good
48	I	-109.762	130.625	14.569	10.189	36.102	0.840	10	12	Good
49	E	-98.687	126.766	15.502	12.032	35.607	0.801	10	12	Good
50	L	-83.009	157.003	18.189	9.587	45.561	0.775	10	12	Good
51	A	-78.909	-31.581	13.345	11.169	43.478	0.762	10	12	Good
52	C	-140.367	152.670	16.846	7.753	45.242	0.791	9	11	Warn
53	G	-101.913	138.716	44.273	21.941	55.336	0.819	10	11	Warn
54	H	-126.742	159.182	27.073	11.749	57.859	0.868	9	11	Warn
55	L	-128.789	154.778	16.941	13.867	55.023	0.895	10	12	Good
56	S	-132.259	140.867	19.974	14.631	42.009	0.914	10	12	Good
57	H	-78.326	122.917	12.811	11.573	51.415	0.919	10	12	Good
58	Q	-55.592	-45.254	5.885	8.167	42.369	0.924	10	12	Good
59	E	-58.891	-36.250	4.054	9.204	36.160	0.920	10	12	Good
60	C	-62.306	-45.644	3.698	6.013	32.869	0.912	10	12	Good
61	L	-65.902	-39.960	5.283	11.177	31.823	0.894	10	12	Good
62	I	-70.538	-36.434	9.991	8.838	29.441	0.875	10	12	Good
63	I	-67.629	-39.038	7.906	6.106	30.124	0.839	10	12	Good
64	S	-67.024	-35.834	8.789	16.218	31.015	0.770	10	12	Good
65	F	-95.973	0.158	10.469	8.671	44.328	0.613	10	11	Good
66	G	81.455	18.780	8.453	10.772	78.178	0.472	10	11	Dyn
67	T	-94.151	141.040	24.778	61.431	46.029	0.350	10	11	Dyn
68	T	-100.742	147.809	25.138	18.099	73.798	0.309	9	8	Dyn
69	?	9999.000	9999.000	0.000	0.000	0.000	0.000	0	6	None
70	K	9999.000	9999.000	0.000	0.000	0.000	0.000	0	6	None
71	A	-48.354	104.260	77.239	62.507	61.102	0.887	6	10	Warn
72	D	-93.926	-14.434	18.969	11.884	55.305	0.816	10	12	Good
73	V	-134.992	157.177	27.614	11.887	48.966	0.745	10	12	Good
74	R	-100.301	123.965	40.448	35.066	44.978	0.668	10	12	Warn
75	A	-63.716	142.625	49.130	22.577	35.272	0.546	10	12	Dyn
76	L	-77.457	142.380	73.923	37.511	41.119	0.472	10	12	Dyn
77	F	-92.063	140.279	24.337	24.968	71.670	0.510	10	10	Dyn
78	P	-66.823	150.634	6.792	8.657	64.678	0.601	10	10	Good
79	F	-108.742	141.297	29.880	17.540	60.676	0.723	10	10	Good
80	C	-97.420	132.950	31.823	26.411	37.815	0.762	10	12	Good
81	T	-63.038	-26.129	6.086	14.558	39.629	0.825	5	12	Warn
82	K	-66.352	-34.796	6.709	12.236	39.972	0.850	10	12	Good
83	C	-66.661	-38.656	9.894	10.386	38.070	0.858	10	12	Good
84	K	-69.425	-32.769	7.449	14.720	71.412	0.803	10	10	Good
85	K	-83.712	-13.845	22.552	24.749	51.926	0.727	10	10	Good
86	D	-103.989	129.776	64.359	31.658	46.568	0.660	10	10	Warn
87	T	-90.805	-14.852	24.031	21.902	65.727	0.496	9	10	Dyn
88	N	-91.994	133.552	71.074	35.686	50.810	0.364	8	10	Dyn

89 K	-92.371	125.879	77.693	15.580	47.857	0.236	7	10	Dyn
90 A	-81.601	128.035	59.209	26.509	76.977	0.156	10	10	Dyn
91 V	-97.817	143.261	37.972	48.478	78.903	0.093	10	10	Dyn
92 Q	9999.000	9999.000	0.000	0.000	0.000	0.000	0	6	None

**Table S3:** Chemical shifts of beta carbons of the cysteine residues coordinating zinc cations. Statistical studies of chemical shifts of  $C_{\beta}$  in Zn-coordinated cysteine residues found a range from 27.5 – 32.5 ppm<sup>[1]</sup>. All the assigned  $C_{\beta}$  resonances in scSte5 RH2 are in this range strongly indicating Zn-coordination.

Cysteine residue	nucleus	$\delta C_{\beta}$ (ppm)
C31	$C_{\beta}$	31.858
C34	$C_{\beta}$	32.525
C52	$C_{\beta}$	32.145
C60	$C_{\beta}$	29.542
C80	$C_{\beta}$	30.547
C83	$C_{\beta}$	30.172

**Table S4:** Backbone resonance assignment (including CB) of scSte5 RH2 in denaturing buffer at 25°C ( see Fig. 4); upon request the authors are happy to provide the chemical shift data in digital form.

Residue	Atom	Nucleus	Shift
S3	CA	13C	58,532
S3	CB	13C	63,82
D4	CA	13C	54,628
D4	CB	13C	41,427
D4	HN	1H	8,159
D4	N	15N	121,61
N5	CA	13C	53,227
N5	CB	13C	38,894
N5	HN	1H	8,358
N5	N	15N	118,575
I6	CA	13C	58,747
I6	CB	13C	38,829
I6	HN	1H	8,061
I6	N	15N	122,572

P8	CA	13C	62,827
P8	CB	13C	32,233
K9	CA	13C	56,626
K9	CB	13C	33,204
K9	HN	1H	8,408
K9	N	15N	121,873
V10	CA	13C	61,841
V10	HN	1H	8,154
V10	N	15N	121,446
P12	CA	13C	63,179
P12	CB	13C	31,969
F13	CA	13C	57,949
F13	CB	13C	39,654
F13	HN	1H	8,216
F13	N	15N	119,966
G14	CA	13C	45,023
G14	HN	1H	8,154
G14	N	15N	110,479
Y15	CA	13C	55,99
Y15	CB	13C	38,4
Y15	HN	1H	7,986
Y15	N	15N	120,951
P16	CA	13C	63,164
P16	CB	13C	31,995
I17	CA	13C	61,319
I17	CB	13C	38,977
I17	HN	1H	8,225
I17	N	15N	121,555
Q18	CA	13C	55,711
Q18	CB	13C	29,639
Q18	HN	1H	8,544
Q18	N	15N	124,898
I22	CA	13C	61,144
I22	CB	13C	38,868
I22	HN	1H	8,225
I22	N	15N	123,079
K23	CA	13C	56,358
K23	CB	13C	33,147
K23	HN	1H	8,413
K23	N	15N	126,224
L27	CA	13C	55,683
L27	CB	13C	42,412
L27	HN	1H	8,171
L27	N	15N	122,638
S30	CA	13C	57,009



S30	CB	13C	65,45
S30	HN	1H	8,477
S30	N	15N	116,108
C31	CA	13C	58,784
C31	CB	13C	31,858
C31	HN	1H	8,532
C31	N	15N	125,192
T32	CA	13C	63,772
T32	CB	13C	68,678
T32	HN	1H	8,513
T32	N	15N	121,139
L33	CA	13C	56,72
L33	CB	13C	43,031
L33	HN	1H	8,913
L33	N	15N	123,651
C34	CA	13C	58,697
C34	CB	13C	32,525
C34	HN	1H	8,027
C34	N	15N	117,729
D35	CA	13C	56,777
D35	CB	13C	40,854
D35	HN	1H	8,061
D35	N	15N	119,397
E36	CA	13C	54,874
E36	CB	13C	29,765
E36	HN	1H	8,583
E36	N	15N	120,474
P37	CA	13C	63,588
P37	CB	13C	32,615
I38	CA	13C	60,956
I38	CB	13C	37,856
I38	HN	1H	8,438
I38	N	15N	124,132
S39	CA	13C	58,607
S39	CB	13C	63,896
S39	HN	1H	8,561
S39	N	15N	117,083
G44	CA	13C	45,59
E45	CA	13C	56,768
E45	CB	13C	30,99
E45	HN	1H	8,04
E45	N	15N	121,195
K46	CA	13C	56,513
K46	CB	13C	33,734
K46	HN	1H	8,645

K46	N	15N	123,987
I47	CA	13C	59,008
I47	CB	13C	38,64
I47	HN	1H	8,677
I47	N	15N	124,439
I48	CA	13C	58,601
I48	CB	13C	39,856
I48	HN	1H	9,081
I48	N	15N	126,043
E49	CA	13C	55,381
E49	CB	13C	30,847
E49	HN	1H	8,607
E49	N	15N	126,416
L50	CA	13C	54,628
L50	CB	13C	43,415
L50	HN	1H	8,858
L50	N	15N	126,453
A51	CA	13C	55,435
A51	CB	13C	18,352
A51	HN	1H	8,939
A51	N	15N	124,043
C52	CA	13C	57,686
C52	CB	13C	32,145
C52	HN	1H	7,403
C52	N	15N	111,688
G53	CA	13C	45,062
G53	HN	1H	7,98
G53	N	15N	111,222
H54	CA	13C	59,542
H54	CB	13C	42,625
H54	HN	1H	8,106
H54	N	15N	120,417
L55	CA	13C	53,967
L55	CB	13C	45,327
L55	HN	1H	9,261
L55	N	15N	118,973
S56	CA	13C	55,916
S56	CB	13C	67,939
S56	HN	1H	8,827
S56	N	15N	111,857
H57	CA	13C	59,164
H57	CB	13C	30,952
H57	HN	1H	9,245
H57	N	15N	125,267
Q58	CA	13C	60,467

Q58	CB	13C	29,13
Q58	HN	1H	9,142
Q58	N	15N	127,537
E59	CA	13C	59,444
E59	CB	13C	29,254
E59	HN	1H	9,742
E59	N	15N	117,481
C60	CA	13C	63,048
C60	CB	13C	29,542
C60	HN	1H	6,794
C60	N	15N	116,168
L61	CA	13C	57,44
L61	CB	13C	42,233
L61	HN	1H	7,38
L61	N	15N	120,584
I62	CA	13C	63,584
I62	CB	13C	37,979
I62	HN	1H	7,865
I62	N	15N	119,536
I63	CA	13C	63,526
I63	CB	13C	38,476
I63	HN	1H	8,084
I63	N	15N	121,364
S64	CA	13C	60,68
S64	CB	13C	63,836
S64	HN	1H	7,94
S64	N	15N	116,029
F65	CA	13C	58,216
F65	CB	13C	39,97
F65	HN	1H	8,189
F65	N	15N	119,46
G66	CA	13C	45,682
G66	HN	1H	8,228
G66	N	15N	109,192
T67	CA	13C	61,753
T67	CB	13C	69,881
T67	HN	1H	8,098
T67	N	15N	112,432
T68	CA	13C	61,882
T68	CB	13C	69,952
T68	HN	1H	8,188
T68	N	15N	114,749
K70	CA	13C	56,854
K70	CB	13C	32,871
A71	CA	13C	52,849

A71	CB	13C	19,369
A71	HN	1H	8,176
A71	N	15N	123,461
D72	CA	13C	54,241
D72	CB	13C	41,393
D72	HN	1H	8,117
D72	N	15N	118,913
V73	CA	13C	63,05
V73	CB	13C	41,392
V73	HN	1H	8,02
V73	N	15N	120,01
R74	CA	13C	56,661
R74	CB	13C	32,488
R74	HN	1H	8,317
R74	N	15N	122,477
A75	CA	13C	52,404
A75	CB	13C	19,395
A75	HN	1H	8
A75	N	15N	123,408
L76	CA	13C	55,122
L76	CB	13C	42,829
L76	HN	1H	7,952
L76	N	15N	120,654
F77	CA	13C	55,441
F77	CB	13C	39,31
F77	HN	1H	8,261
F77	N	15N	120,661
P78	CA	13C	63,221
P78	CB	13C	31,8
F79	CA	13C	56,897
F79	CB	13C	40,638
F79	HN	1H	7,897
F79	N	15N	118,689
C80	CA	13C	59,324
C80	CB	13C	30,547
C80	HN	1H	8,489
C80	N	15N	123,078
T81	CA	13C	64,69
T81	CB	13C	69,087
T81	HN	1H	8,341
T81	N	15N	118,64
K82	CA	13C	58,192
K82	CB	13C	32,882
K82	HN	1H	8,37
K82	N	15N	123,468

C83	CA	13C	63,356
C83	CB	13C	30,172
C83	HN	1H	8,196
C83	N	15N	121,896
K84	CA	13C	57,02
K84	CB	13C	32,943
K84	HN	1H	7,863
K84	N	15N	120,059
K85	CA	13C	56,671
K85	CB	13C	33,368
D86	CA	13C	54,494
D86	CB	13C	41,37
D86	HN	1H	8,397
D86	N	15N	121,554
T87	CA	13C	62,13
T87	CB	13C	69,736
T87	HN	1H	8,128
T87	N	15N	113,995
N88	CA	13C	53,525
N88	CB	13C	38,991
K89	CA	13C	56,411
K89	CB	13C	33,206
K89	HN	1H	8,208
K89	N	15N	121,885
A90	CA	13C	52,64
A90	CB	13C	19,303
A90	HN	1H	8,332
A90	N	15N	125,493
V91	CA	13C	62,373
V91	CB	13C	32,944
Q92	CA	13C	57,56
Q92	CB	13C	30,623
Q92	HN	1H	8,012
Q92	N	15N	128,627

**Table S5:** Peak list of 3D  $^{15}\text{N}$  resolved [ $^1\text{H}$ - $^1\text{H}$ ]-NOESY of scSte5 RH2 in denaturing buffer at 25°C.

<b>Residue</b>	<b>w1</b>	<b>w2</b>	<b>w3</b>	<b>Peak Intensity</b>
N5	4,704	118,6	8,35	846202
	5,794	118,6	8,35	83197
	8,345	118,6	8,34	174697
I6	1,856	122,5	8,05	407622
	4,453	122,5	8,04	214113
	8,047	122,5	8,05	5931914
	5,796	122,5	8,05	148912
	1,492	122,5	8,05	179746
	1,146	122,5	8,05	164946
	4,701	122,5	8,05	4052652
	4,619	122,6	8,05	80823
K9	4,703	121,9	8,41	2213701
	4,444	121,9	8,41	311524
V10	2,017	121,4	8,15	255285
	1,789	121,4	8,15	93950
	0,916	121,4	8,15	334354
	4,285	121,4	8,15	963973
	8,153	121,4	8,15	2506919
	4,703	121,5	8,15	3322393
	5,792	121,5	8,15	212210
F13	4,704	120,0	8,22	1665894
	4,545	120,0	8,21	89680
	4,359	120,0	8,21	219672
	5,794	120,0	8,21	143739
	3,049	120,0	8,21	110012
G14	4,704	110,5	8,16	400856
Y15	3,759	120,9	7,99	178025
	4,704	120,9	7,99	2262362
	3,831	120,9	7,99	149558
	2,843	121,0	7,99	154474
	5,791	121,0	7,98	179028
	3,046	121,0	7,99	93532
I17	2,242	121,5	8,22	145675
	1,821	121,6	8,22	686153
	0,908	121,6	8,22	238470
	4,14	121,6	8,22	256937
	8,223	121,6	8,22	6916294
	1,525	121,6	8,22	265436
	1,211	121,6	8,22	282161
	4,703	121,6	8,22	2729326

	5,79	121,7	8,23	131456
Q18	5,795	124,9	8,54	82501
	4,141	124,9	8,54	126686
	4,704	124,9	8,54	920009
I22	4,705	123,1	8,22	803765
	5,798	123,1	8,22	90583
K23	4,173	126,2	8,41	100425
	4,704	126,2	8,41	681817
	5,788	126,2	8,41	76924
S30	4,705	116,1	8,48	306954
C31	1,978	125,2	8,53	259090
	4,803	125,2	8,53	312659
	3,469	125,2	8,53	579173
	0,291	125,2	8,53	141219
	4,258	125,2	8,53	137551
	0,825	125,2	8,53	73317
	8,531	125,2	8,53	996028
	8,044	125,2	8,53	82975
	0,719	125,2	8,53	75591
	4,53	125,2	8,53	124029
T32	4,159	121,1	8,51	155667
	8,511	121,1	8,51	640868
	4,358	121,1	8,51	160458
	5,724	121,2	8,51	107240
	4,258	121,2	8,51	347538
	1,27	121,2	8,51	282298
	0,289	121,2	8,51	81363
	8,907	121,2	8,51	143079
L33	1,272	123,6	8,91	153661
	8,519	123,6	8,91	168647
	1,758	123,6	8,91	430644
	8,907	123,6	8,91	1230294
	8,032	123,6	8,91	466726
	0,897	123,6	8,91	355553
	1,543	123,6	8,91	192128
	4,254	123,6	8,9	81019
	3,125	123,6	8,91	73500
	4,689	123,6	8,91	251483
C34	3,366	117,7	8,03	174232
	1,541	117,7	8,03	163449
	1,975	117,7	8,03	83220
	0,897	117,7	8,03	186729
	4,997	117,7	8,03	246579
	3,457	117,7	8,02	159694

	8,56	117,7	8,02	122836
	3,133	117,7	8,03	236226
	4,63	117,7	8,03	170673
	8,907	117,7	8,03	477891
	8,034	117,7	8,03	1830193
	1,76	117,7	8,03	274990
D35	4,997	119,4	8,06	125105
	8,91	119,4	8,06	110898
	3,467	119,4	8,06	226597
	2,747	119,4	8,06	203109
	4,614	119,4	8,06	756433
	8,053	119,4	8,06	1928572
	3,053	119,4	8,06	211124
	8,575	119,4	8,06	328198
E36	3,923	120,5	8,58	75145
	1,974	120,5	8,58	122109
	2,474	120,5	8,58	329763
	4,615	120,5	8,58	213164
	8,056	120,5	8,58	393488
	2,215	120,5	8,58	328098
	8,578	120,5	8,58	2466285
	2,312	120,5	8,58	593060
	4,842	120,5	8,58	266991
	3,469	120,5	8,58	260143
	7,317	120,5	8,58	134740
	3,743	120,5	8,58	74401
	3,051	120,5	8,58	83292
I38	4,706	124,1	8,44	337184
	3,468	124,1	8,44	96138
	1,523	124,1	8,65	82040
	0,826	124,1	8,44	139002
	4,802	124,1	8,43	218020
	1,228	124,1	8,43	103213
	2,394	124,1	8,44	89375
	8,436	124,1	8,44	717448
	0,712	124,1	8,43	189314
	4,53	124,1	8,44	389999
	1,844	124,1	8,44	81040
	1,619	124,1	8,44	252589
	0,29	124,2	8,44	100168
	3,951	124,2	8,44	114514
S39	4,704	117,1	8,56	188070
E45	5,789	121,1	8,04	86651
	8,041	121,2	8,04	661830
	4,706	121,2	8,04	1270252



	4,161	121,2	8,04	147098
	4,401	121,2	8,04	204182
	2,133	121,2	8,04	228852
	2,043	121,2	8,04	252575
	3,824	121,2	8,04	122113
	2,367	121,2	8,04	145724
K46	2,36	124,0	8,64	77811
	4,403	124,0	8,64	275774
	4,704	124,0	8,64	632807
I47	1,52	124,3	8,67	80762
	1,788	124,4	8,67	275915
	1,388	124,4	8,68	228098
	0,764	124,4	8,68	230065
	4,506	124,4	8,67	593893
	8,675	124,4	8,67	911477
	1,912	124,4	8,68	298959
I48	4,416	126,0	9,07	158155
	1,19	126,0	9,07	191206
	0,755	126,0	9,07	735466
	1,911	126,1	9,07	123920
	4,587	126,1	9,07	500197
	1,805	126,1	9,07	316417
	8,825	126,1	9,07	143650
	9,07	126,1	9,07	1253198
	1,32	126,1	9,07	165735
E49	4,699	126,4	8,61	80333
	0,752	126,4	8,61	658852
	8,606	126,4	8,61	1746497
	4,415	126,4	8,61	866304
	4,579	126,4	8,6	169852
	1,785	126,4	8,61	444894
	1,988	126,4	8,61	369710
	2,281	126,4	8,6	87080
L50	2,007	126,5	8,86	137696
	0,736	126,5	8,86	313071
	1,877	126,5	8,86	130151
	4,583	126,5	8,86	414521
	4,457	126,5	8,86	152195
	2,275	126,5	8,86	250400
	8,854	126,5	8,86	881677
	5,207	126,5	8,86	100135
	0,828	126,5	8,86	385509
	1,675	126,5	8,86	309948
	1,769	126,5	8,86	158740
A51	1,788	124,1	8,65	240892

C52	3,369	111,7	7,41	193368
	0,836	111,7	7,41	200211
	2,738	111,7	7,4	251772
	2,269	111,7	7,4	153794
	7,406	111,7	7,4	2371302
	8,107	111,7	7,4	132113
	4,425	111,7	7,41	418520
	1,682	111,7	7,4	74532
	1,385	111,7	7,41	358175
	4,04	111,7	7,41	144630
	1,879	111,7	7,4	146946
	7,98	111,7	7,4	552966
	3,667	111,7	7,4	83741
G53	4,04	111,2	7,98	80107
	1,385	111,2	7,98	86373
	7,406	111,2	7,98	480016
	7,98	111,2	7,98	2084353
	4,239	111,2	7,98	503445
	3,663	111,2	7,98	598405
	8,104	111,2	7,98	396679
	4,425	111,2	7,98	180737
	2,278	111,2	7,98	129636
	0,836	111,2	7,98	76203
H54	4,336	120,3	8,11	132198
	4,24	120,4	8,1	158157
	3,662	120,4	8,1	127012
	2,265	120,4	8,1	104370
	9,257	120,4	8,1	74076
	1,27	120,4	8,1	84464
	3,293	120,4	8,1	216516
	8,106	120,4	8,1	2102443
	7,981	120,4	8,1	366801
	0,842	120,4	8,1	180014
	3,157	120,4	8,1	330425
	1,676	120,4	8,1	85863
L55	3,159	119,0	9,25	139180
	1,616	119,0	9,26	199554
	9,255	119,0	9,26	909906
	1,082	119,0	9,25	111613
	3,289	119,0	9,26	174045
	5,206	119,0	9,26	86518
	4,685	119,0	9,26	434192
	1,269	119,0	9,26	134975
	0,737	119,0	9,26	375799
	1,706	119,0	9,25	293295

	7,301	119,0	9,26	89287
	8,831	119,0	9,26	77140
	0,83	119,0	9,26	98815
S56	1,083	111,9	8,82	178548
	9,25	111,9	8,82	111550
	5,208	111,9	8,82	540745
	1,69	111,9	8,82	142798
	1,616	111,9	8,82	153070
	9,069	111,9	8,82	168000
	8,824	111,9	8,82	1585862
	4,584	111,9	8,82	132191
	1,796	111,9	8,82	126064
	0,294	111,9	8,82	89935
	0,74	111,9	8,82	687089
	3,572	111,9	8,82	235180
	3,433	111,9	8,83	76898
	5,718	111,9	8,82	109859
H57	0,811	125,3	9,24	107129
	4,015	125,3	9,24	156112
	0,29	125,3	9,24	107846
	9,239	125,3	9,24	1077052
	4,738	125,3	9,24	109217
	3,435	125,3	9,24	165111
	5,72	125,3	9,24	415066
	3,564	125,3	9,24	199150
	0,888	125,3	9,24	74938
	3,795	125,3	9,24	112992
Q58	0,778	127,5	9,14	208073
	1,785	127,5	9,13	89555
	2,262	127,5	9,14	298655
	9,135	127,5	9,13	671350
	3,795	127,5	9,13	81577
	2,348	127,5	9,14	196746
	4,726	127,5	9,14	387754
	4,591	127,5	9,13	110220
	2,015	127,5	9,13	225387
	3,927	127,5	9,14	188326
	9,731	127,5	9,14	101191
E59	2,099	117,5	9,73	235487
	2,264	117,5	9,73	136326
	2,035	117,5	9,73	251518
	9,734	117,5	9,73	470255
	4,087	117,5	9,73	167632
	2,35	117,5	9,73	274223
	4,706	117,5	9,74	276731

C60	9,736	116,1	6,8	113038
	4,715	116,1	6,8	78797
	4,015	116,1	6,8	370193
	2,983	116,1	6,8	172067
	2,041	116,1	6,8	121574
	3,114	116,2	6,8	144914
	2,353	116,2	6,8	92986
	7,384	116,2	6,8	225802
	6,802	116,2	6,8	819788
	3,793	116,2	6,8	144029
	1,763	116,2	6,8	75039
	0,879	116,2	6,8	97420
	L61	6,8	120,6	7,38
3,114		120,6	7,39	73386
3,996		120,6	7,38	380781
1,751		120,6	7,38	367659
1,615		120,6	7,38	489887
2,986		120,6	7,38	116312
7,383		120,6	7,38	1429510
0,831		120,6	7,38	356879
7,867		120,6	7,38	270402
0,898		120,6	7,39	227474
I63	8,086	121,4	8,08	1766650
	1,849	121,4	8,09	496789
	1,602	121,4	8,09	450577
	4,007	121,4	8,09	113655
	0,857	121,4	8,09	733169
	7,867	121,4	8,09	254829
	1,924	121,4	8,09	273303
	3,918	121,4	8,08	324592
	3,809	121,4	8,08	248951
	1,172	121,4	8,09	305475
S64	3,515	116,0	7,94	117425
	3,705	116,0	7,94	131857
	3,922	116,0	7,94	138589
	4,287	116,0	7,94	139484
	8,087	116,0	7,94	107682
	1,848	116,0	7,94	118530
	7,94	116,1	7,94	352889
	4,704	116,1	7,94	655042
	0,86	116,1	7,94	197548
F65	8,192	119,6	8,19	139480
	8,197	119,7	8,19	142255
G66	4,705	109,2	8,23	523812
T67	4,707	112,5	8,1	106811

T68	4,704	114,7	8,2	92546
A71	4,705	123,3	8,18	589685
D72	2,641	118,9	8,12	84226
	4,25	118,9	8,11	122809
	5,793	118,9	8,12	77201
	4,703	119,0	8,12	833581
V73	4,048	120,0	8,02	230748
	2,642	120,0	8,02	126086
	2,138	120,0	8,02	343821
	4,617	120,0	8,02	606672
	0,937	120,0	8,02	729713
	4,704	120,0	8,02	1625315
	8,018	120,0	8,02	1756265
R74	5,794	122,4	8,32	86626
	4,226	122,5	8,31	92908
	4,703	122,5	8,32	1042173
	1,766	122,5	8,32	115920
	4,049	122,5	8,31	186214
	0,938	122,5	8,31	104479
	1,63	122,5	8,32	96654
	1,832	122,5	8,31	100100
A75	4,703	123,4	8	1104000
	5,791	123,4	8	82300
	8,001	123,4	8	242662
	1,322	123,4	8	288803
	4,234	123,4	8	300492
L76	8,255	120,7	7,95	111518
	1,319	120,7	7,95	464152
	4,246	120,7	7,95	790875
	1,486	120,7	7,95	534724
	0,832	120,7	7,95	131291
	7,956	120,7	7,95	1130974
F77	0,788	120,6	8,26	116840
	4,805	120,6	8,26	191563
	3,018	120,7	8,26	197128
	8,261	120,7	8,26	1341774
	2,879	120,7	8,26	266703
	1,488	120,7	8,26	176099
	4,232	120,7	8,26	642379
	1,318	120,7	8,26	215768
	7,206	120,7	8,26	121415
	7,953	120,7	8,26	91522
	4,704	120,7	8,26	834099
F79	7,897	118,7	7,9	1013462

	4,373	118,7	7,9	517416
	2,004	118,7	7,9	98338
	2,976	118,7	7,9	196841
	4,7	118,7	7,9	418116
	3,059	118,7	7,9	177250
	1,748	118,7	7,9	125129
	7,156	118,7	7,9	117720
C80	4,701	123,0	8,48	448806
	8,484	123,1	8,48	108658
	2,939	123,1	8,48	86289
	3,066	123,1	8,48	120250
T81	4,354	118,6	8,34	184438
	1,349	118,6	8,34	125070
K82	4,102	123,5	8,37	82488
	1,242	123,5	8,37	358261
	4,214	123,5	8,37	209169
	8,195	123,5	8,37	330962
	8,368	123,5	8,37	1036777
	1,337	123,5	8,37	254609
	4,348	123,5	8,37	163169
	1,504	123,5	8,37	179768
	4,704	123,6	8,37	356567
C83	1,505	121,9	8,19	143278
	1,234	121,9	8,19	232980
	1,724	121,9	8,41	92480
	8,195	121,9	8,2	1950635
	8,366	121,9	8,19	435704
	3,058	121,9	8,19	309550
	2,756	121,9	8,19	290760
	7,864	121,9	8,19	96878
	4,262	121,9	8,19	355082
	2,915	121,9	8,19	102103
	1,351	121,9	8,19	137550
K84	1,81	120,0	7,86	277371
	4,184	120,1	7,86	234780
	4,703	120,1	7,86	910889
	8,192	120,1	7,86	160747
	4,264	120,1	7,86	203579
	1,391	120,1	7,86	125454
	8,093	120,1	7,86	74205
	1,809	120,1	7,87	275512
	1,61	120,1	7,86	122302
T87	4,704	114,0	8,13	371594
A90	1,392	125,5	8,33	78380
	8,317	125,5	8,32	84302

	5,791	125,5	8,33	141454
	4,302	125,5	8,33	88506
	4,704	125,5	8,33	1422396
Q92	2,293	128,6	8,01	75631
	1,38	128,7	8,01	125631
	1,935	128,7	8,01	247950
	2,086	128,7	8,01	222728
	4,704	128,7	8,01	767630
	4,122	128,7	8,01	1644399
	0,953	128,7	8,01	281219
	0,34	128,7	8,01	85194

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