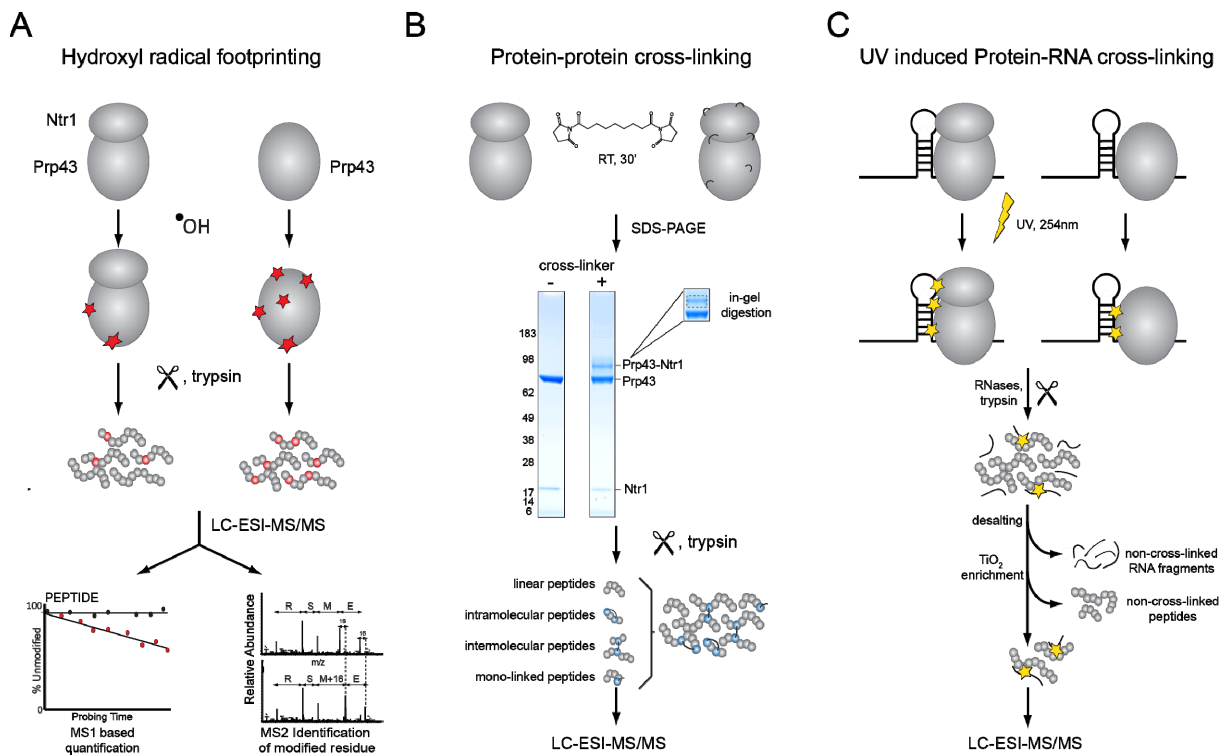
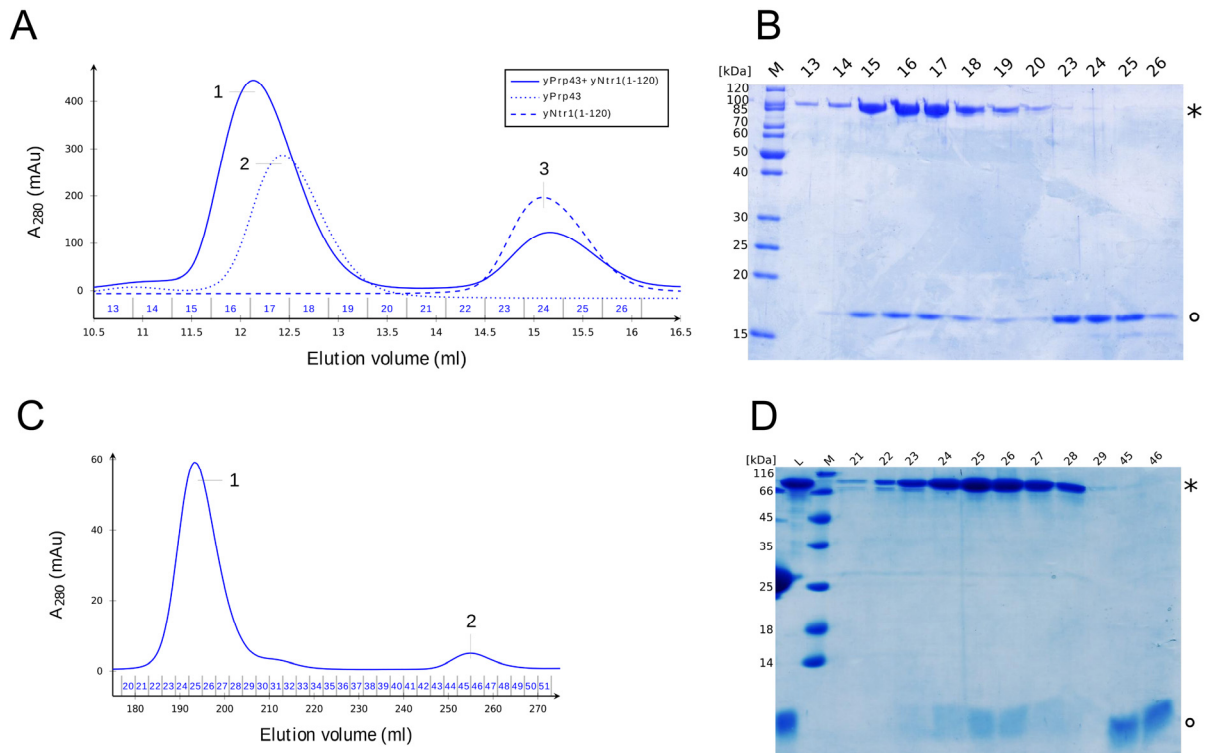


Supplementary Figures



Supplementary Figure S1. Hydroxyl radical footprinting, protein-protein cross-linking and protein-RNA cross-linking experimental workflows.

A) Free and bound Prp43 were treated with hydroxyl radicals and digested into peptides prior to LC-MS analysis. Modified peptides were identified and quantified, resulting in dose-response plots. From the relative levels of modification inferences as to relative solvent accessibility between free Prp43 and Ntr1 bound Prp43 can be made. B) The complex Prp43/Ntr1(1-120) was treated with increasing molar excesses of BS3 for 30 minutes to determine optimal cross-linking reaction conditions. In parallel experiments, the complexes Prp43/Ntr1(1-120) and Prp43/Ntr1(51-110) were treated with the determined molar excess of BS3 and BS2G for 30 minutes at room temperature. The band corresponding to the MW of the complex was excised and in-gel digested and directly injected for LC-MS analysis. C) Prp43/RNA and Prp43/Ntr1(120)/RNA were subjected to UV induced protein-RNA cross-linking. Proteins and RNA were hydrolyzed and the resulting mixtures enriched for protein-RNA heteroconjugates. For this purpose, the samples were treated with reverse-phase desalting for the removal of non-cross-linked oligonucleotides, and subsequently with TiO_2 chromatography for the removal of non-cross-linked peptides. The enriched samples were then subjected to LC-ESI-MS/MS analysis.



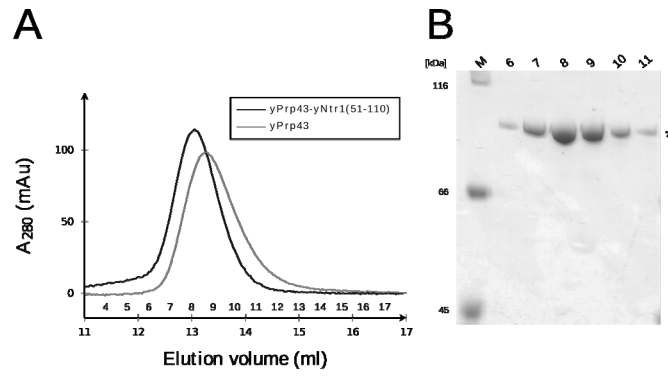
Supplementary Figure S2. Complex formation of Prp43/Ntr1(1-120) and Prp43/Ntr1(51-110).

A) Chromatogram of the analytical size exclusion chromatography of the complex Prp43/Ntr1(1-120), using a Superdex 200 (10/300) column. The absorption at 280 nm (machine specific unit, y-axis) is shown with respect to the elution volume (ml, x-axis) for three independent samples: Prp43+Ntr1(1-120) (continuous line), free Prp43 (dotted line) and free Ntr1(1-120) (dashed line). The local maxima correspond to the Prp43/Ntr1(1-120) complex (1), Prp43 (2) and Ntr1(1-120) (3). The fractions indicated along the x-axis have been collected during the run of Prp43 and Ntr1(1-120).

B) Coomassie-stained 15 % SDS-PA gel of the analytical S200 size exclusion chromatography of Prp43 and Ntr1(1-120). The marker (M) and elution fractions (numbers) from the gel filtration are shown on the lanes. The position of Prp43 is labeled by an asterisk, that of Ntr1(1-120) by a circle.

C) Chromatogram of the size exclusion chromatography of Prp43 and Ntr1(51-110), using a Superdex 200 26/60 column. The blue line reflects the UV absorption at 280 nm (machine specific unit, y-axis) with respect to the elution volume (ml, x-axis). The numbers of collected fractions are indicated along the x-axis. The first local maximum (1) corresponds to the Prp43/Ntr1(51-110) complex and the second (2) to Ntr1(51-110), which was added in excess.

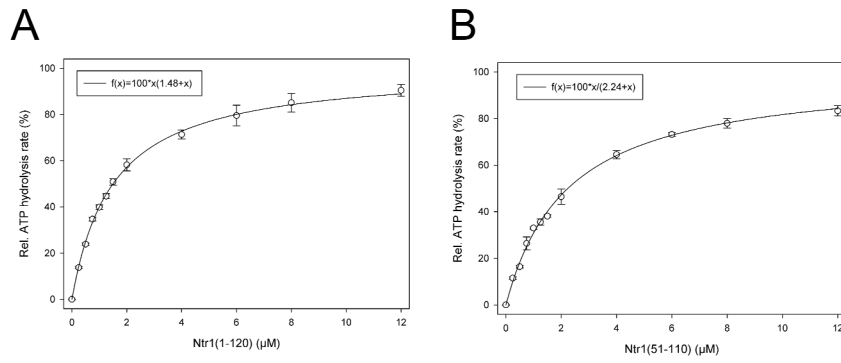
D) Coomassie-stained 17.5 % SDS-PA gel of the S200 size exclusion chromatography of the complex of Prp43 and Ntr1(51-110). The lanes contain the loaded sample (L), the molecular weight marker (M) and samples collected during the chromatography (numbers). The position of Prp43 is indicated by an asterisk, the position of Ntr1(51-110) by a circle.



Supplementary Figure S3. Analytical size exclusion chromatography of the fusion protein Prp43-Ntr1(51-110) in comparison to Prp43

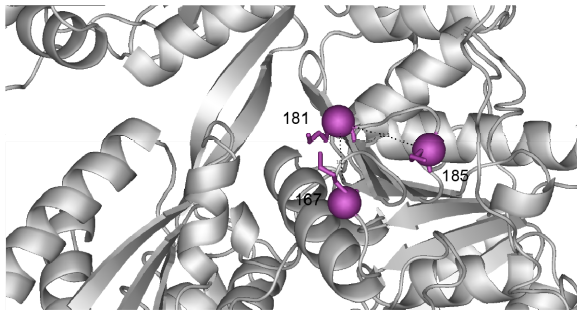
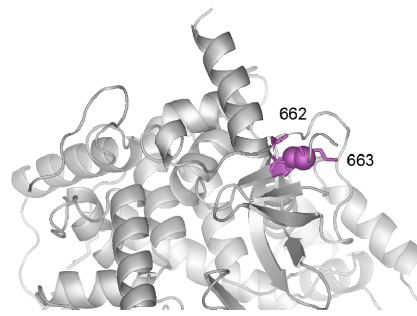
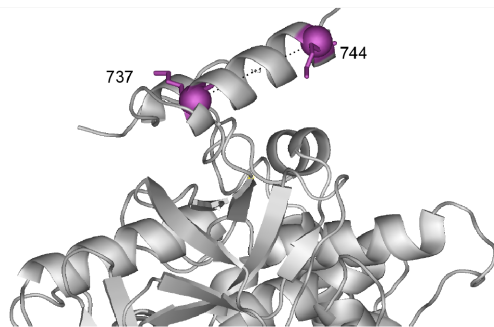
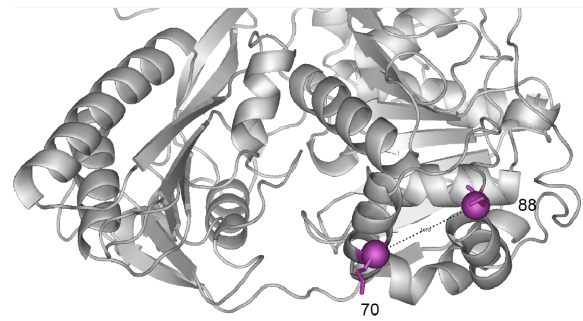
A) The chromatogram shows the absorption at 280 nm in a machine specific unit (mAu, y-axis) with respect to the elution volume (ml, x-axis). The fusion protein Prp43-Ntr1(51- 110) is shown in black, Prp43 in grey. The collected fractions from the run of the fusion protein are indicated along the x-axis.

B) Coomassie-stained 7.5 % SDS-PA gel showing the molecular weight marker (M) and elution fractions (numbers) collected during the gel filtration of Prp43-Ntr1(51-110). The position of the fusion protein is indicated by an asterisk.



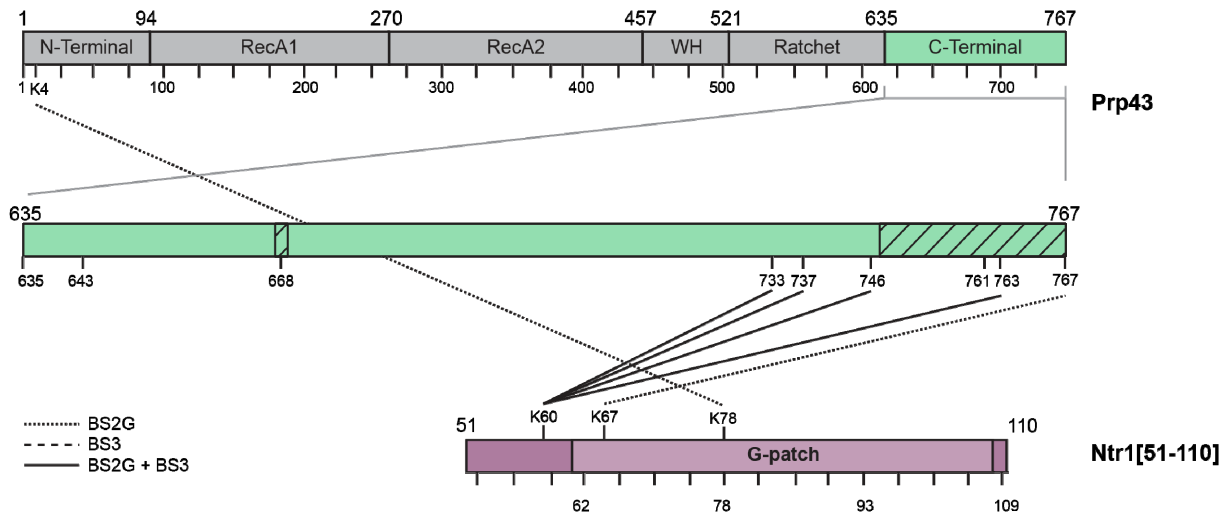
Supplementary Figure S4. Determination of the dissociation constants of the complexes of Prp43 with Ntr1(1-120) and Ntr1(51-110).

The relative ATPase activity (y-axis) is plotted against the concentration of the Ntr1-fragment (x-axis). The K_d for the complex Prp43/Ntr1(1-120) is $1.49 \pm 0.05 \mu\text{M}$ and $2.24 \pm 0.11 \mu\text{M}$ for the complex Prp43/Ntr1(51-110). The symbols indicate the rel. ATPase activity for the respective concentrations of the Ntr1-fragment, the error bars the root mean square deviation and the continuous line the hyperbolic fit. The equation of the fit is provided in the figure legend.

A**B****C****D**

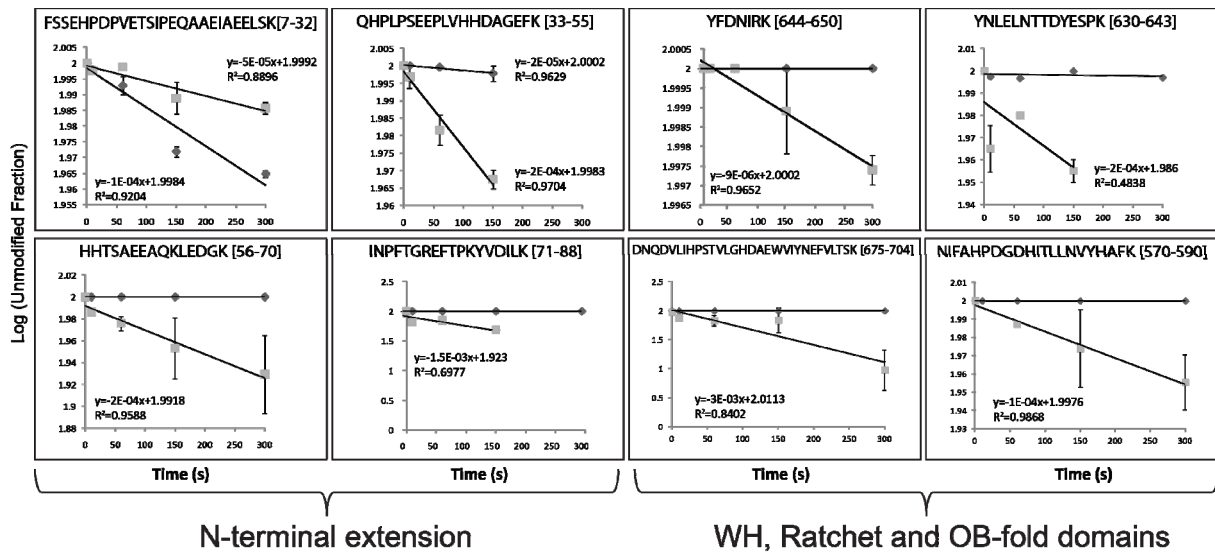
Supplementary Figure S5. Prp43 intra-molecular cross-links.

Intra-protein cross-links identified in Prp43 that can be mapped onto the available crystal structure are shown. All mapped cross-links are within the maximum distance that the cross-linker can span. Panel A depicts cross-linked pairs 167-181 and 181-185 in the RecA1 domain. Panel B shows residues 662-663 in the C-terminal domain, whereas panel C shows residues 737-744 of the same domain. Lastly, panel D depicts residues 70-80 in the N-terminal domain.



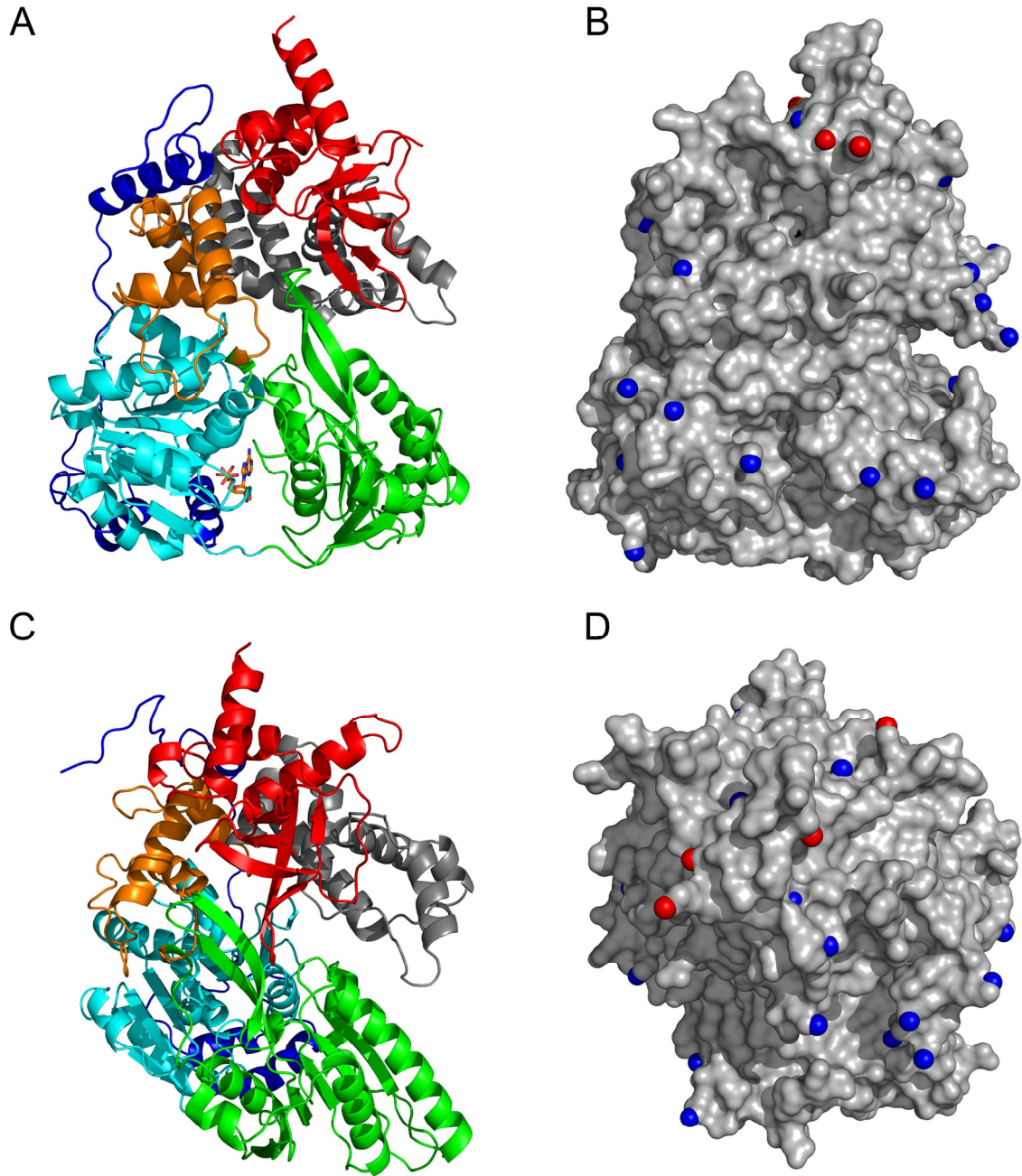
Supplementary Figure S6. Protein-Protein cross-linking map of the Prp43/Ntr1(51-110) complex

Cross-linking map depicting inter-protein cross-links of Prp43/Ntr1(51-110). Shaded areas in Prp43 indicate regions missing in the crystal structure. The cross-linking pattern of this shorter fragment is similar to the longer 1-120 fragment, with residues K60 and K67 contacting the most C-terminal region of Prp43. In addition, there is a contact between K4 at the very N-terminus and K78 of Ntr1 that was not observed in the longer fragment.



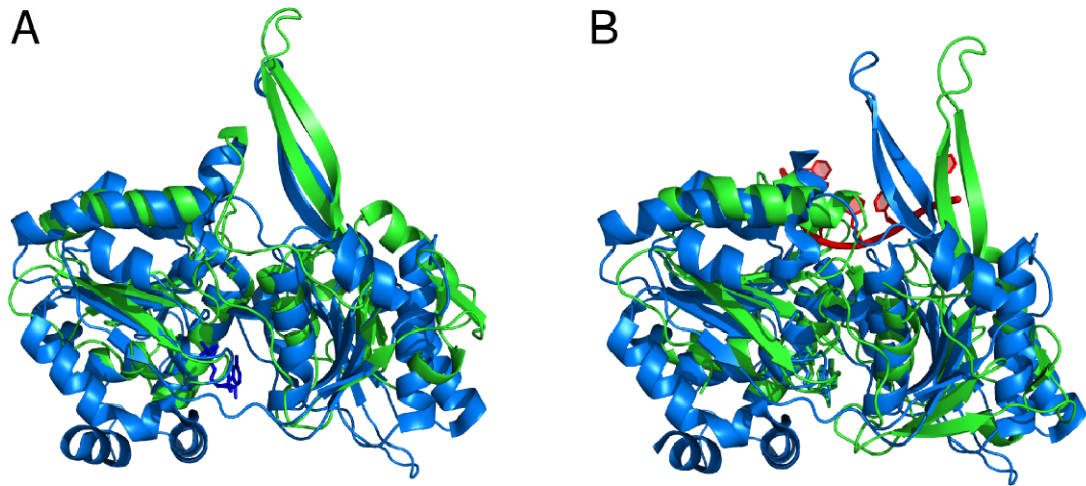
Supplementary Figure S7. Dose-Response curves from hydroxyl radical footprinting

Dose response plots show the levels of oxidative modification attained in the unbound and in the Ntr1 bound Prp43. The N-terminal domain shows an interesting protection pattern, with protection on the α N1 helix that connects the top of the structure and increased solvent accessibility along the structured loop that connects the α N1 helix with the α N2 and α N3 helices on the bottom of the structure. The biggest differences observed in the N-terminal domain (left side) correspond to peptides (56-70) and (71-88) that can be mapped to the α N2 and α N3 helices. Peptide (7-32), on the other hand, shows a moderate protection from modification upon Ntr1 binding. The C-terminal domain is more accessible to oxidative modification upon Ntr1 binding, with residues W693, H579 and H587 exhibiting the highest levels of modification.



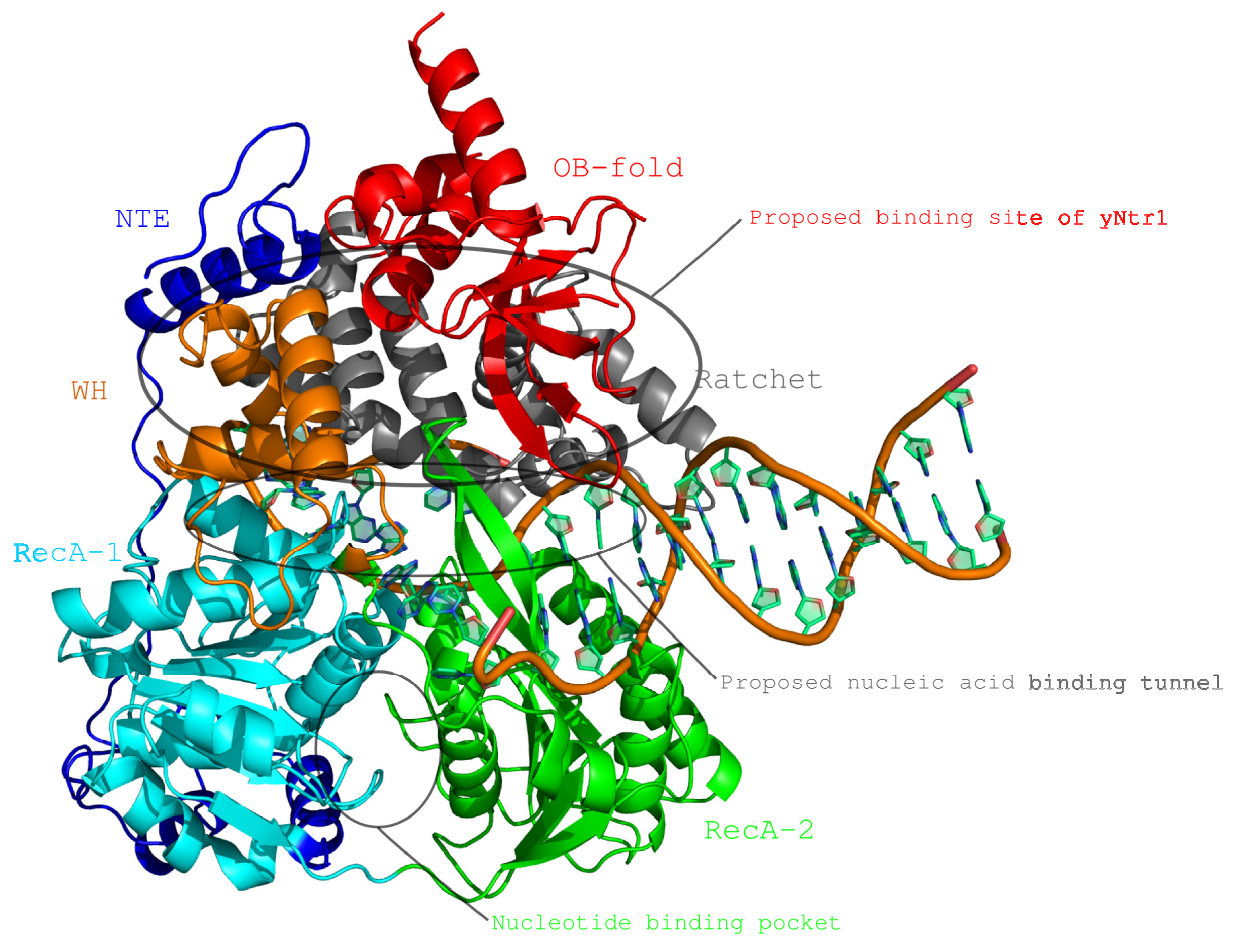
Supplementary Figure S8. Localization of the lysine residues cross-linked to Ntr1 within the crystal structure of Prp43.

The structure of Prp43 is shown in front view (A+B) and top view (C+D) in the cartoon (A+C) and in the surface (B+D) representation. Within the surface representations, lysine residues which have been cross-linked to Ntr1 are colored in red, all other lysine residues are colored in blue (pdb-ID for Prp43: 2xau).



Supplementary Figure S9: Superposition of the helicase core domains of Prp43 (marine blue) and the HCV NS3 helicase (green).

For Prp43 (pdb-ID 2xau), the ADP-bound state is shown in both subfigures, whereas for the NS3 helicase, (A) provides the ADP bound state (pdb-ID 3o8b) and (B) the state with a bound ATP analog and poly(T)₆-DNA, colored in red (pdb-ID 3kql).



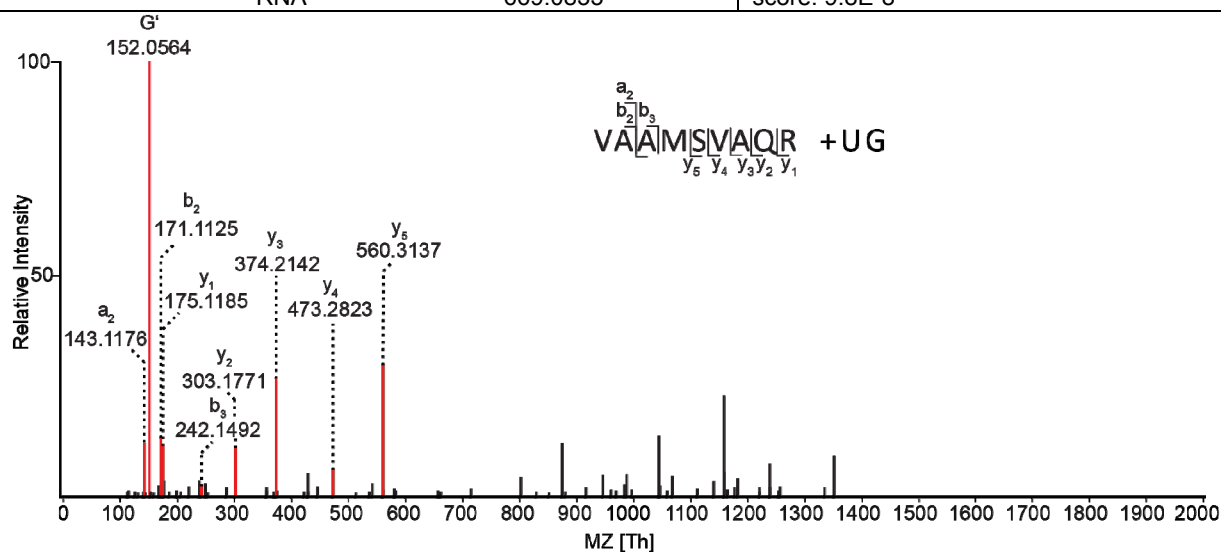
Supplementary Figure S10: Structure of Prp43 (pdb-ID 2xau) superimposed with the dsDNA substrate from the structure of the Ski2-like helicase Hel308 (pdb-ID 2p6r).

The domains of Prp43 are labeled in the respective color and the nucleotide binding pocket, the proposed nucleic acid binding tunnel and the putative binding site of the G-patch motif of Ntr1 is indicated.

Supplementary Figure S11. Protein-RNA cross-links spectra. For each spectrum, a short table is provided and the cross-linked amino acid is indicated in bold, whereas red letters indicate that the corresponding residue is oxidized. Raw refers to experimentally determined parameters in the corresponding Thermo Scientific *.RAW file; m/z refers to mass to charge ratio, M(exp) corresponds to the experimentally determined mass of the cross-link, RT to the retention time, and scan index to the number of the spectrum in the corresponding file. DB stands for database, m(calc) refer to the calculated masses of the RNA moiety, peptide and combination of both (XL). In each spectrum, the corresponding b and y ions are indicated. These refer to ions which retain the charge on the N terminus or C-terminus, respectively. RNA marker ions are indicated with the corresponding letter (ie. G',U'). Mass shifts in the sequence tags help identify the site of cross-linking and are indicated for the corresponding fragments: 52 refers to C3O, a CID generated fragment of uracil; 94 refers to U'-H2O.

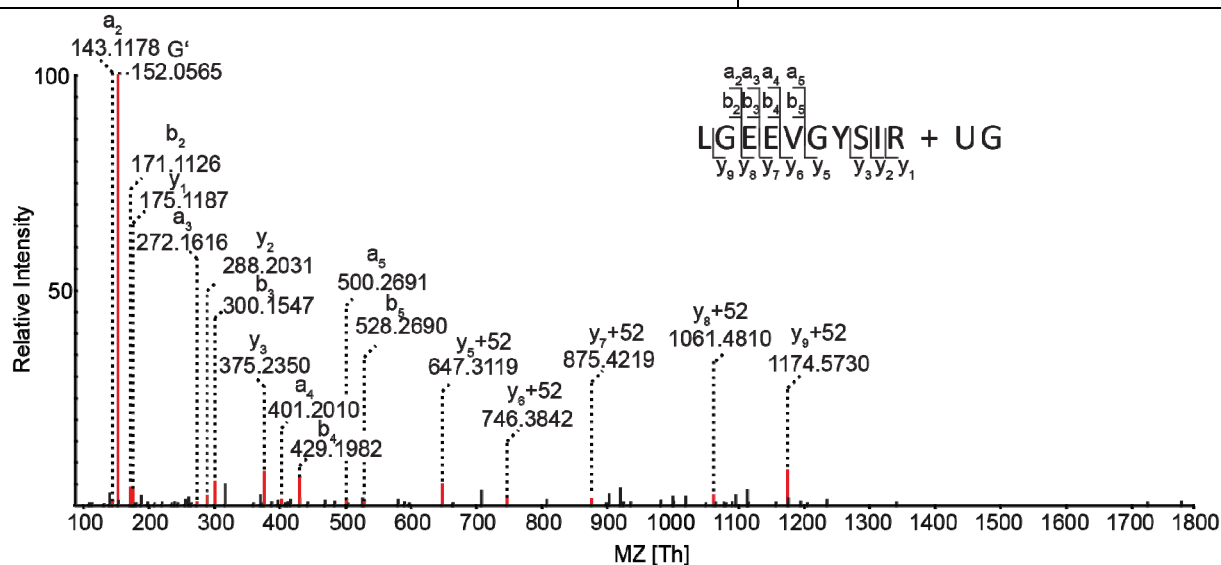
Prp43: VAAM**S**VQAQR+UG

| | | | | |
|----------|-----------------|---------------------|------------------|--------------------|
| Raw | m/z 801.2943 | M(exp) 1600.5728 | RT (min) 19.8 | Scan Index 1899 |
| m (calc) | XL | 1600.5736 | DB: | Prp43-Ntr1 |
| | Peptide | 931.4903 | Search Engine: | OMSSA |
| | RNA | 669.0833 | score: 9.8E-8 | |



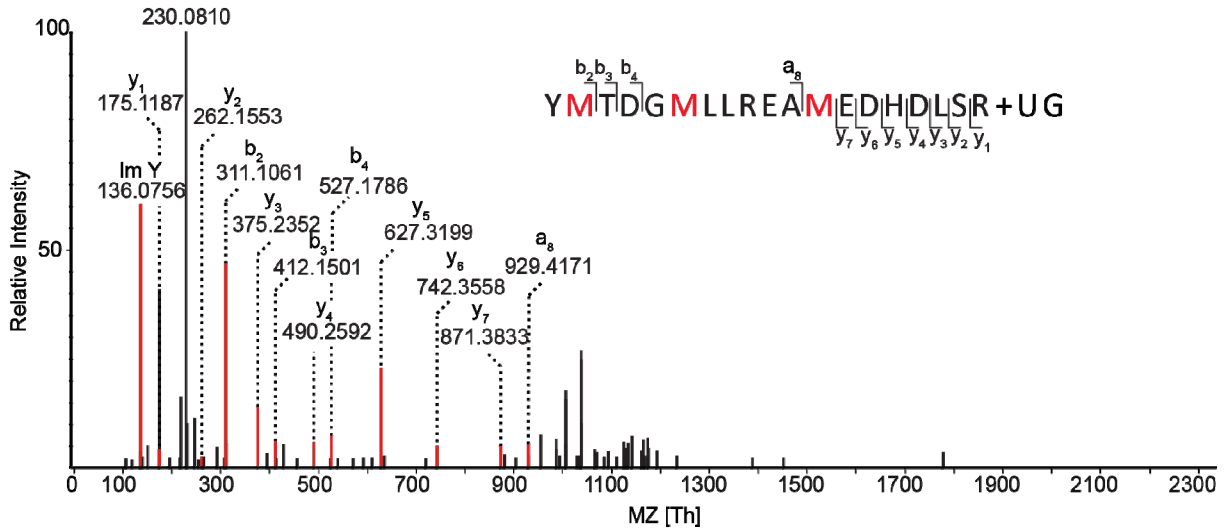
Prp43: LGEEV**G**YSIR+UG

| | | | | |
|----------|-----------------|---------------------|------------------|--------------------|
| Raw | m/z 597.8918 | M(exp) 1790.6517 | RT (min) 25.7 | Scan Index 2453 |
| m (calc) | XL | 1790.6544 | DB: | Prp43-Ntr1 |
| | Peptide | 1121.5711 | Search Engine: | OMSSA |
| | RNA | 669.0833 | score: 1.4e-5 | |



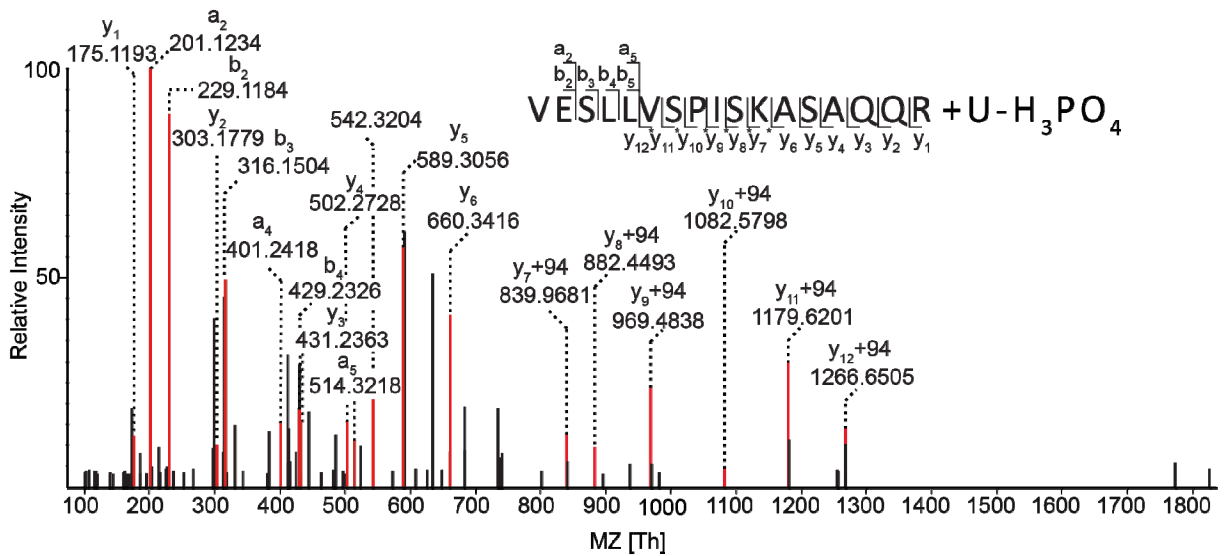
Prp43: YM(OX)TDGM(OX)LLREAM(OX)EDHDLRSR+U

| | m/z | M(exp) | RT (min) | Scan Index |
|----------|----------|-----------|-----------------|------------|
| Raw | 885.6848 | 2654.0307 | 28.9 | 2748 |
| m (calc) | XL | 2654.0330 | DB: | Prp43-Ntr1 |
| | Peptide | 2329.9971 | Search Engine: | OMSSA |
| | RNA | 324.0359 | score: 3.71E-11 | |



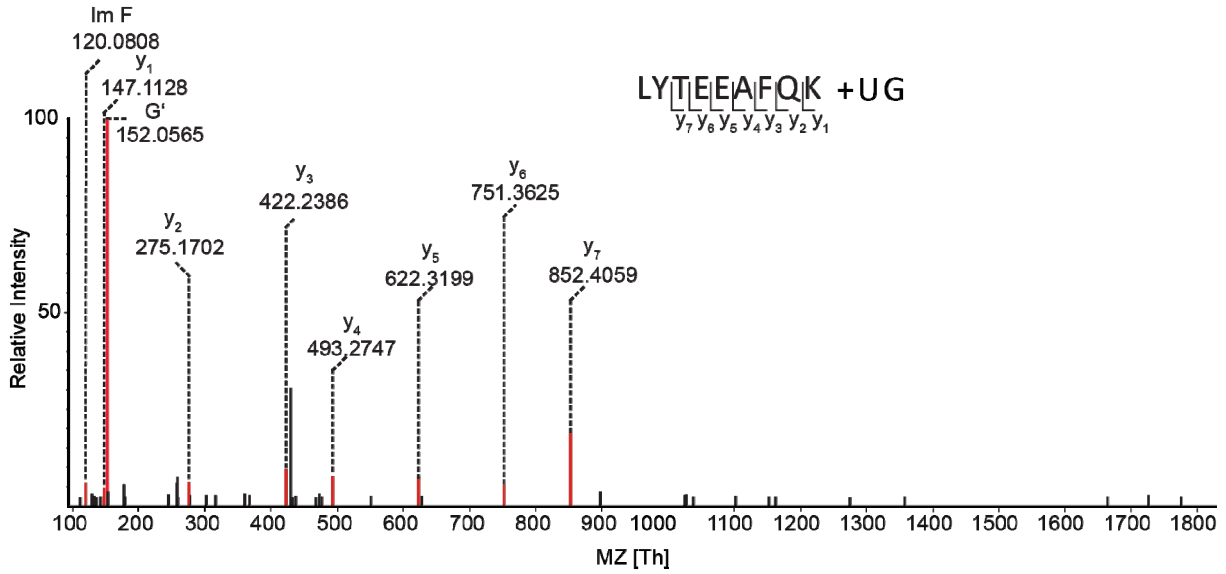
Prp43: VESLLVSPISKASAQQR + U - H₃PO₄

| | m/z | M(exp) | RT (min) | Scan Index |
|----------|----------|-----------|----------------|------------|
| Raw | 733.6743 | 2197.9992 | 32.2 | 3058 |
| m (calc) | XL | 2198.0015 | DB: | Prp43-Ntr1 |
| | Peptide | 1812.0099 | Search Engine: | OMSSA |
| | RNA | 385.9916 | score: 8.5E-11 | |



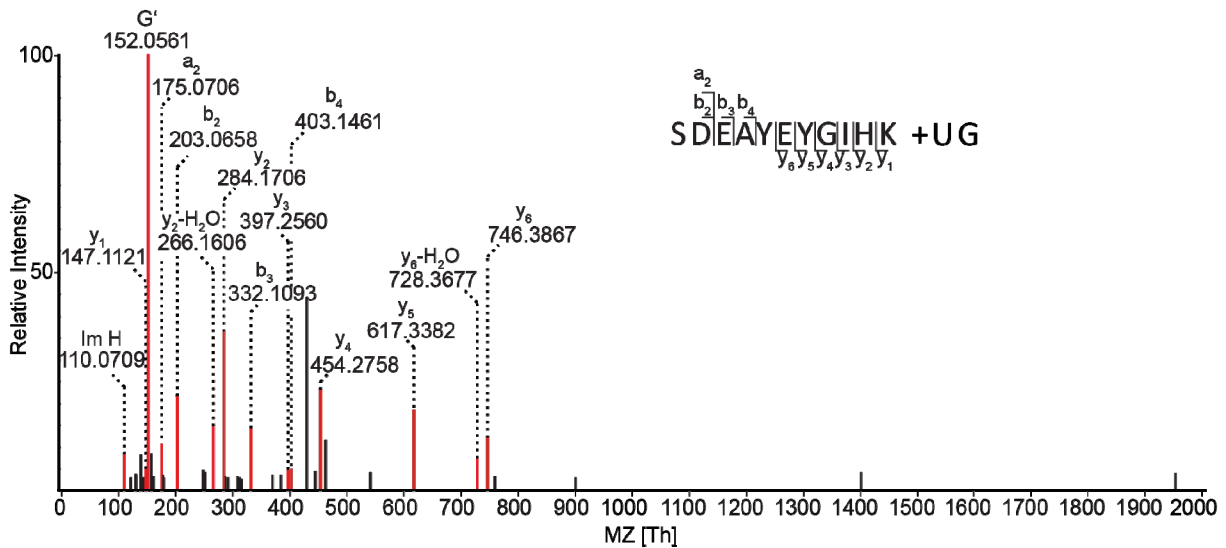
Prp43: LYTEEFQK+UG

| | m/z | M(exp) | RT (min) | Scan Index |
|----------|----------|-----------|----------------|------------|
| Raw | 899.3226 | 1796.6294 | 25.50 | 2436 |
| m (calc) | XL | 1796.6326 | DB: | Prp43-Ntr1 |
| | Peptide | 1127.5493 | Search Engine: | OMSSA |
| | RNA | 669.0833 | Score: 5.6E-6 | |



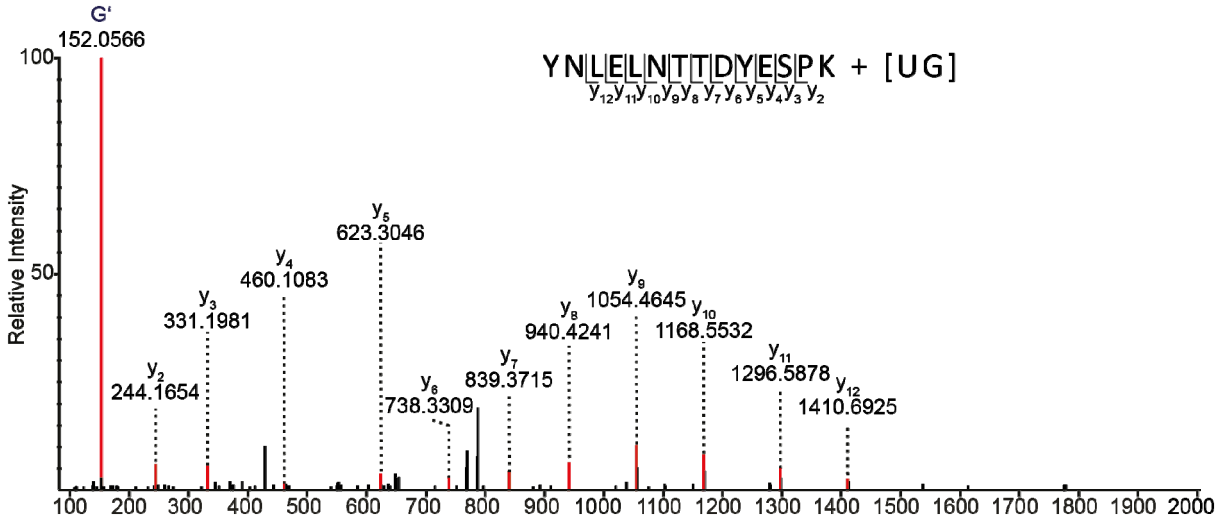
Prp43: SDEAYEYGIHK+UG

| | m/z | M(exp) | RT (min) | Scan Index |
|----------|----------|-----------|-----------------|------------|
| Raw | 990.8375 | 1979.6592 | 22.98 | 2189 |
| m (calc) | XL | 1979.6606 | DB: | Prp43-Ntr1 |
| | Peptide | 1310.5773 | Search Engine: | OMSSA |
| | RNA | 669.0833 | Score: 4.01E-11 | |



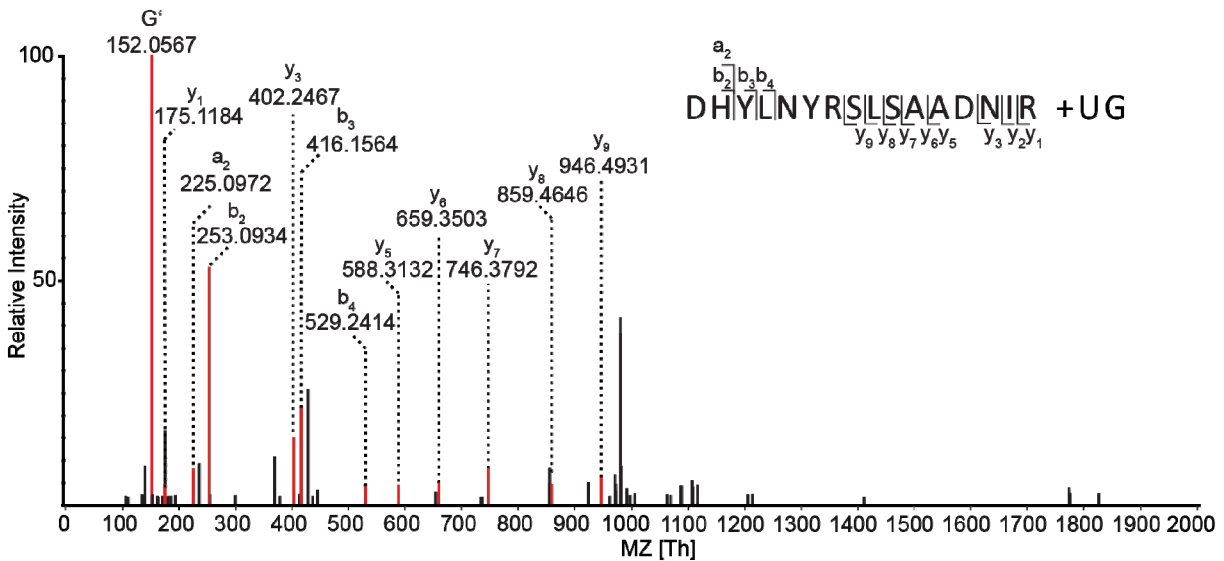
Prp43: YNLELNTTIDYESPK + GU

| Raw | m/z | M(exp) | RT (min) | Scan Index |
|----------|----------|-----------|-----------------|------------|
| Raw | 785.9604 | 2354.8575 | 29.3 | 2734 |
| m (calc) | XL | 2354.8611 | DB: | Prp43-Ntr1 |
| | Peptide | 1685.7778 | Search Engine: | OMSSA |
| | RNA | 669.0833 | Score: 3.91E-15 | |



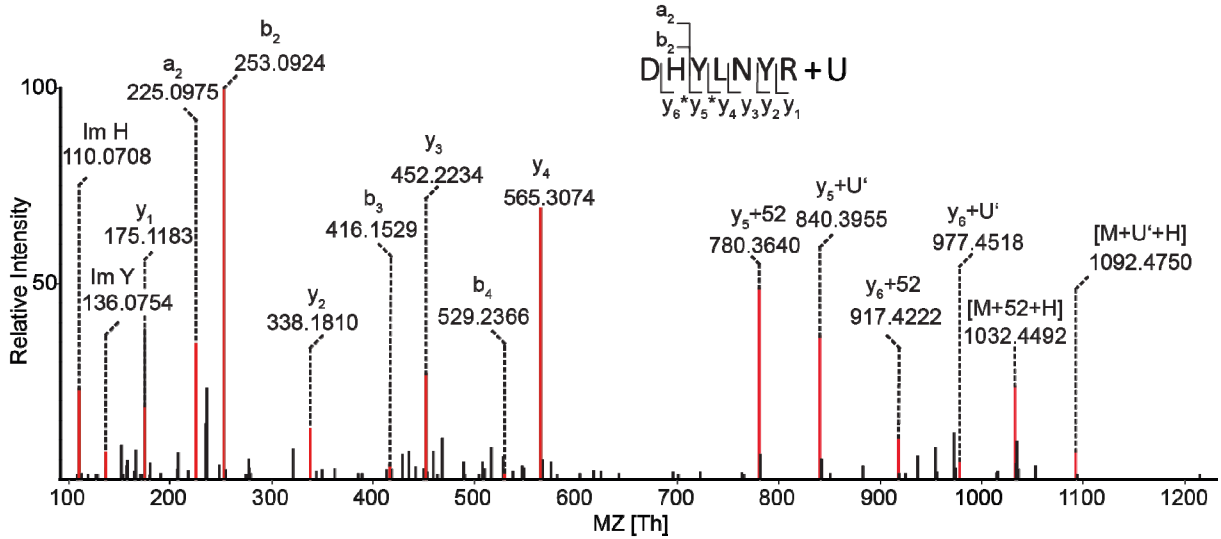
Prp43: DHYLNYSLSAADNIR+UG

| Raw | m/z | M(exp) | RT (min) | Scan Index |
|----------|----------|-----------|-----------------|------------|
| Raw | 859.6778 | 2576.0097 | 30.7 | 2927 |
| m (calc) | XL | 2576.0112 | DB: | Prp43-Ntr1 |
| | Peptide | 1907.9358 | Search Engine: | OMSSA |
| | RNA | 669.0833 | Score: 2.59E-12 | |



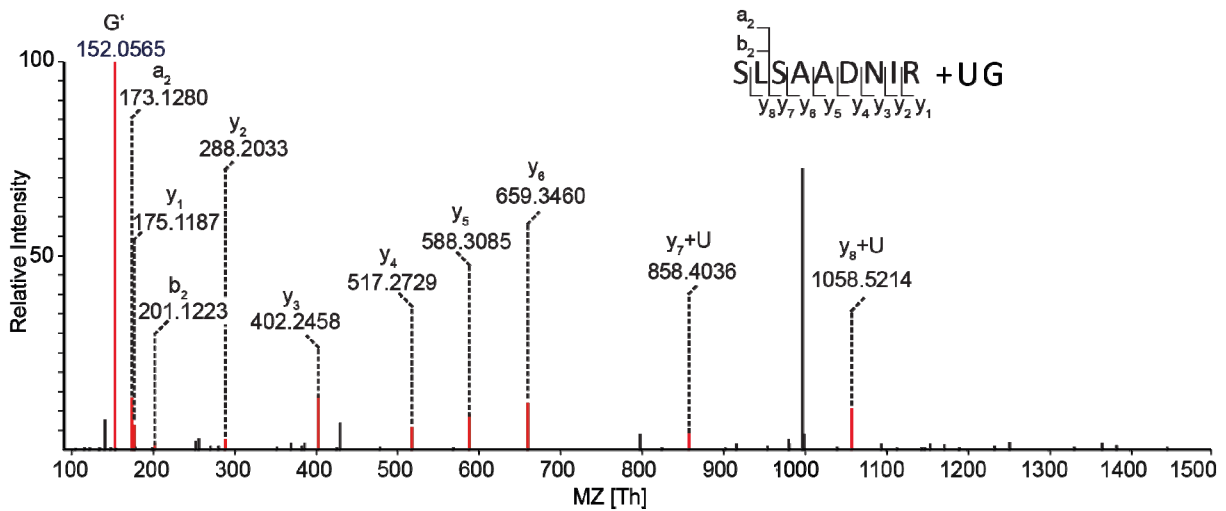
Prp43: DHYLN^UYR+U

| | m/z | M(exp) | RT (min) | Scan Index |
|----------|----------|-----------|----------------|------------|
| Raw | 652.7503 | 1303.4848 | 20.49 | 1955 |
| m (calc) | XL | 1303.4865 | DB: | Prp43-Ntr1 |
| | Peptide | 979.4506 | Search Engine: | OMSSA |
| | RNA | 324.0359 | 5.4E-6 | |



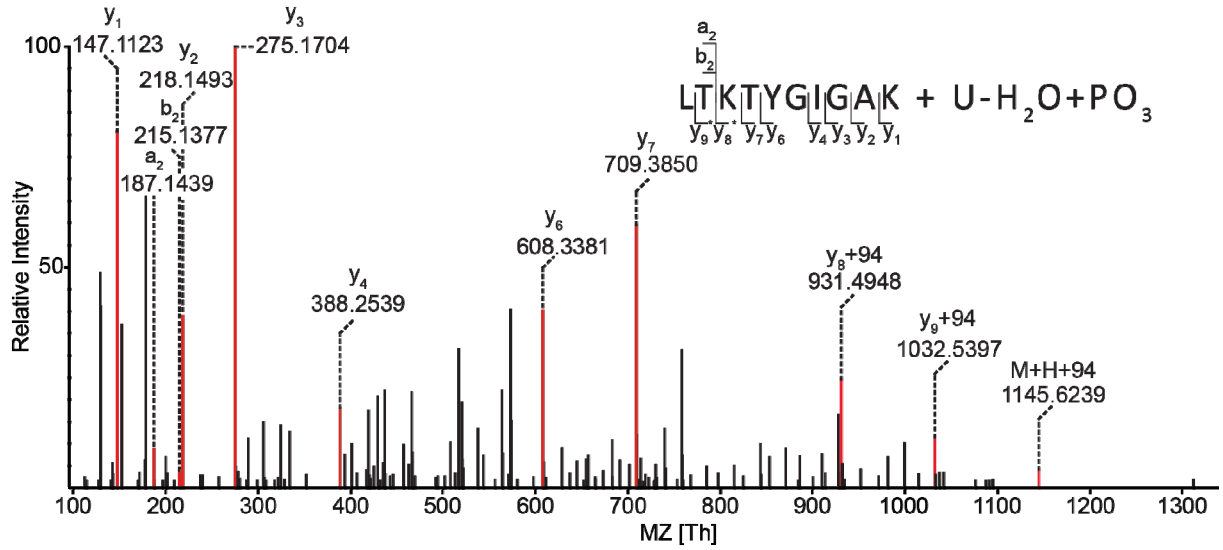
Prp43: SL^USAADNIR+UG

| | m/z | M(exp) | RT (min) | Scan Index |
|----------|----------|-----------|----------------|------------|
| Raw | 808.2924 | 1614.5690 | 23.89 | 2270 |
| m (calc) | XL | 1614.5706 | DB: | Prp43-Ntr1 |
| | Peptide | 945.4873 | Search Engine: | OMSSA |
| | RNA | 669.0833 | 3.32E-8 | |



Ntr1(1-120): LTKTYGIGAK+U-H₂O+PO₃

| | m/z | M(exp) | RT (min) | Scan Index |
|----------|----------|-----------|----------------|------------|
| Raw | 719.3062 | 1436.5966 | 22.83 | 2169 |
| m (calc) | XL | 1436.5983 | DB: | Prp43-Ntr1 |
| | Peptide | 1050.6067 | Search Engine: | OMSSA |
| | RNA | 385.9916 | 6.7E-6 | |



Supplementary Table S1. Sequences of the DNA oligonucleotides.

The sequences of all oligonucleotides used during molecular cloning are provided in this table.

| Name | Sequence (5'-3') |
|-----------------------|--|
| Prp43-for | AATGGGTTCCAAAAGAAGATTCTCGTCCGAAC |
| Prp43-rev | TCCCTTTCTTGGAGTGCTTACTCTTCTTTTTG |
| Ntr1-for | AATGGAGGATTCCGACTCCAACACAGATAAAAAG |
| Ntr1(120)-rev | TCCCATCCTCATTTTCACTGTGATAGTTACTGGAG |
| Ntr1(51)-for | AATGAATGCCCAACGATCTCAAAATTAACGAAG |
| Ntr1(110)-rev | TCCCGTTGGTATTTGAAAACATTCTAGACCTGC |
| Prp43-IF-for | AGGAGATATACCATGGGCGTTCCAAAAGAAGATTCTCGTCCGAACAC |
| Prp43-IF-51-rev | GATCGTTGGGGCATTTCCTTGGAGTGCTTACTCTTCTTTTTGTTTTACCTTG |
| Prp43-745-IF-51-rev | GATCGTTGGGGCATTTCCTTGATCCTTTCCAAAGATAATTTGACATCACC |
| Prp43-IF-59-rev | ACCATATGTCTTCGTTTTCTTGGAGTGCTTACTCTTCTTTTTGTTTTACCTTG |
| Prp43-IF-62-rev | CGCACCAATACCATATTTCTTGGAGTGCTTACTCTTCTTTTTGTTTTACCTTG |
| Ntr1-51-FL-for | AAGCACTCCAAGAAAAATGCCCAACGATCTCAAAATTAACGAAGACATATG |
| Ntr1-51-745-for | GAAAGGATCAAGGAAAATGCCCAACGATCTCAAAATTAACGAAGACATATG |
| Ntr1-59-FL-for | AAGCACTCCAAGAAAACGAAGACATATGGTATTGGTGCGAAGTTAC |
| Ntr1-62-FL-for | AAGCACTCCAAGAAATATGGTATTGGTGCGAAGTTACTTTTCGAGTATGG |
| Ntr1-110-rev | CCGCAAGCTTGTTCGATCATTTTTCGAACTGCGGGTGGCTCCAGTTGGTATTTGAAAACATTCTAGACCTGCATTG |
| Ntr1-102-rev | CCGCAAGCTTGTTCGATCATTTTTCGAACTGCGGGTGGCTCCAAAACATTCTAGACCTGCATTGTGCATAGG |
| Ntr1-99-rev | CGCAAGCTTGTTCGATCATTTTTCGAACTGCGGGTGGCTCCAGTGCATAGGTCGGCTTTGGGTTTCTATTGG |
| Ntr1_K67E-for | GTATTGGTGCGGAGTTACTTTTCGAGTATGGG |
| Ntr1_K67E-rev | CCCATACTCGAAAGTAACTCCGCACCAATAC |
| Prp43_K418E-for | GTTTCCCCTATCTCCGAGGCTTCTGCCCAACAAAGAGCTG |
| Prp43_K418E-rev | CAGCTCTTTGTTGGGCAGAAGCCTCGGAGATAGGGGAAAC |
| Prp43_Y595A-for | CAAATCGGATGAAGCTGCTGAATATGGTATCCATAAGTG |
| Prp43_Y595A-rev | CACTTATGGATACCATATTCAGCAGCTTCATCCGATTTG |
| Prp43_Y610A_S614A-for | GCCGTGACCACTATCTAAATGCCAGGTCCCTTGCCGCTGCTG |
| Prp43_Y610A_S614A-rev | CAGCAGCGGCAAGGGACCTGGCATTAGATAGTGGTCACGGC |
| Prp43_Y630A-for | CCCAATTAGAAAAGATTAATGAACCGTGCCAACCTAGAATTAAC |
| Prp43_Y630A-rev | GTTTAATTCTAGGTTGGCACGGTTCATTAATCTTTCTAATTGGG |

Supplementary Table S2. Intra-molecular cross-linking spectra identified in Prp43. Cross-linked lysines are indicated in bold, the specific positions are specified in the residue column. Whenever present, OxiM indicates hydroxylation of methionine. BS2G refers to bis(sulfosuccinimidyl)glutarate whereas BS3 refers to bis(sulfosuccinimidyl)suberate. MM Score indicates the score obtained from mass matrix with the specific search parameters whereas C-C refers to the C alpha distance of the lysine pair in the published crystal structure (pdb 2XAU).

| # | Peptide | Residue (Prp43) | Crosslinker | MM Score | C-C |
|----|---|-----------------|-------------|----------|------|
| 1 | VAEEMDV K LGEEVGYSIR FEN K TSNK + OxiM(5) | K181-K167 | BS2G | 30.00 | 10.7 |
| 2 | SGA K GYITVK D K KR | K668-K562 | BS2G | 83.34 | |
| 3 | ALASGFFMQVA K KR + OxiM(8) | K663-K662 | BS2G | 86.28 | 2.4 |
| 4 | YNLELNTTDYESP K YFDNIR LNEL K QGK | K754-K643 | BS2G | 59.84 | |
| 5 | K ALASGFFMQVAK LNEL K QGK + OxiM(9) | K754-K650 | BS2G | 66.88 | |
| 6 | YNLELNTTDYESP K YFDNIR QG K NK | K757-K643 | BS2G | 104.34 | |
| 7 | K ALASGFFMQVAK QG K NK + OxiM(9) | K757-K650 | BS2G | 123.72 | |
| 8 | K ALASGFFMQVAK N K K + OxiM(9) | K759-K650 | BS2G | 120.61 | |
| 9 | YNLELNTTDYESP K YFDNIR K SK | K761-K643 | BS2G | 72.35 | |
| 10 | K ALASGFFMQVAK K SK | K761-K650 | BS2G | 131.74 | |
| 11 | YNLELNTTDYESP K YFDNIR S K HSK | K763-K643 | BS2G | 31.70 | |
| 12 | K ALASGFFMQVAK S K HSK | K763-K650 | BS2G | 91.49 | |
| 13 | YNLELNTTDYESP K YFDNIR H S KK | K766-K643 | BS2G | 33.20 | |
| 14 | LNEL K QGK I K EK | K754-K744 | BS2G | 57.41 | |
| 15 | LNEL K QGK E K VDR | K754-K746 | BS2G | 121.13 | |
| 16 | I K E K VDR QG K NK | K757-K746 | BS2G | 65.40 | |
| 17 | LNEL K QG K NK | K757-K754 | BS2G | 63.03 | |
| 18 | I K E K VDR N K K | K759-K746 | BS2G | 44.35 | |
| 19 | LNEL K QGK N K K | K759-K754 | BS2G | 145.37 | |
| 20 | LNEL K QGK K SK | K761-K754 | BS2G | 97.67 | |
| 21 | I K E K VDR K SK | K761-K746 | BS2G | 47.00 | |
| 22 | I K E K VDR S K HSK | K763-K746 | BS2G | 54.89 | |
| 23 | GDV K LSLER S K HSK | K763-K737 | BS2G | 43.65 | |
| 24 | LNEL K QGK S K HSK | K763-K754 | BS2G | 119.45 | |
| 25 | LNEL K QGK H S KK | K767-K754 | BS2G | 66.18 | |
| 26 | LNEL K QGK H S KK | K767-K754 | BS3 | 74.95 | |
| 27 | K ALASGFFMQVAK H S KK | K766-K650 | BS3 | 60.35 | |
| 28 | LNEL K QGK S K HSK | K763-K754 | BS3 | 45.49 | |
| 29 | K ALASGFFMQVAK S K HSK | K763-K650 | BS3 | 45.3 | |
| 30 | K ALASGFFMQVAK K SK | K761-K650 | BS3 | 59.18 | |
| 31 | I K E K VDR K SK | K761-K746 | BS3 | 29.7 | |
| 32 | LNEL K QGK K SK | K761-K754 | BS3 | 55.84 | |
| 33 | LNEL K QGK N K K | K759-K754 | BS3 | 95.04 | |
| 34 | I K E K VDR N K K | K759-K746 | BS3 | 34.9 | |
| 35 | K ALASGFFMQVAK N K K | K759-K650 | BS3 | 89.8 | |
| 36 | K ALASGFFMQVAK QG K NK | K757-K650 | BS3 | 43.91 | |
| 37 | LNEL K QGK E K VDR | K754-K746 | BS3 | 137.06 | |
| 38 | K ALASGFFMQVAK LNEL K QGK | K754-K650 | BS3 | 82.26 | |
| 39 | GDV K LSLER I K EK | K744-K737 | BS3 | 49.84 | 14.3 |
| 40 | ALASGFFMQVA K KR | K663-K662 | BS3 | 85.63 | 2.4 |
| 41 | FEN K TSN K TILK | K185-K181 | BS3 | 35.75 | 11.1 |
| 42 | LEDG K INPFTGR YVDIL K IR | K88-K70 | BS3 | 15.45 | 16.5 |

Supplementary Table S3. Inter-protein cross-links identified in Prp43-Ntr1(1-120). Cross-linked lysines are indicated in bold, the specific positions are specified in the residue column. Whenever present, OxiM indicates hydroxylation of methionine. BS2G refers to bis(sulfosuccinimidyl)glutarate whereas BS3 refers to bis(sulfosuccinimidyl)suberate.

| # | Peptide (Prp43) | Peptide (Ntr1(1-120)) | Residue (Prp43) | Residue Ntr1 | crosslinker |
|----|--|------------------------------|-----------------|--------------|-------------|
| 1 | HSK K | TYGIGAK K LLSSMGYVAGK | K767 | K67 | BS2G |
| 2 | S K H S K | TYGIGAK K LLSSMGYVAGK | K763 | K67 | BS2G/BS3 |
| 3 | HSK K | LT K TYGIGAK | K767 | K60 | BS2G |
| 4 | S K H S K | LT K TYGIGAK | K763 | K60 | BS2G |
| 5 | K SK | LT K TYGIGAK | K761 | K60 | BS2G |
| 6 | IKE K VDR | LT K TYGIGAK | K746 | K60 | BS2G |
| 7 | TVTSVRPEWLIEIAPAYYDLSNFQ K GDVK | LT K TYGIGAK | K733 | K60 | BS2G |
| 8 | SGA K GYITVK | K FFFK | K668 | K11 | BS2G |
| 9 | E K VDR | K FFFK | K746 | K11 | BS2G |
| 10 | YNLELN T TDYES P K YFDNIR | FFF K K | K643 | K15 | BS2G |
| 11 | IKE K VDR | FFF K K | K746 | K15 | BS2G |
| 12 | SGA K GYITVK | FFF K K | K668 | K15 | BS3 |
| 13 | GDV K L S L S L R | FFF K K | K737 | K15 | BS3 |
| 14 | IKE K VDR | FFF K K | K746 | K15 | BS3 |
| 15 | GDV K L S L S L R | LT K TYGIGAK | K737 | K60 | BS3 |

Supplementary Table S4. Inter-protein cross-links identified in Prp43/Ntr1(51-110). Cross-linked lysines are indicated in bold, the specific positions are specified in the residue column. Whenever present, OxiM indicates hydroxylation of methionine. BS2G refers to bis(sulfosuccinimidyl)glutarate whereas BS3 refers to bis(sulfosuccinimidyl)suberate.

| # | Peptide (Prp43) | Peptide (Ntr1(51-110)) | Residue (Prp43) | Residue Ntr1 | crosslinker |
|---|---|------------------------------|-----------------|--------------|-------------|
| 1 | TVTSVRPEWLI EIAPAYYDLSN FQ KGDVK | LT K TYGIGAK | K733 | K60 | BS3/BS2G |
| 2 | GDV K LSLER | LT K TYGIGAK | K737 | K60 | BS3/BS2G |
| 3 | IKE K VDR | LT K TYGIGAK | K746 | K60 | BS3/BS2G |
| 4 | S K HSK | LT K TYGIGAK | K763 | K60 | BS3/BS2G |
| 5 | HS K K | TYGIGAK L LLSSMGYVAGK | K766 | K67 | BS2G |
| 6 | GS K R | LLSSMGYVAG K GLGK | K4 | K78 | BS2G |