

**Supplementary material for the manuscript:**

**The redox-sensitive R-loop of the carbon control protein SbtB contributes to the regulation of the cyanobacterial CCM**

**Oliver Mantovani<sup>1</sup>, Michael Haffner<sup>2</sup>, Peter Walke<sup>1</sup>, Abdalla A. Elshereef<sup>2,3</sup>, Berenike Wagner<sup>2</sup>, Daniel Petras<sup>2</sup>, Karl Forchhammer<sup>2</sup>, Khaled A. Selim<sup>2,4,5\*</sup>, Martin Hagemann<sup>1,6\*</sup>**

1 – Institute of Biosciences, Department of Plant Physiology, University of Rostock, Rostock, Germany

2 – Interfaculty Institute of Microbiology and Infection Medicine Tübingen, University of Tübingen, Tübingen, Germany

3 - Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, Egypt

4 – Department of Protein Evolution, Max Planck Institute for Biology, Tübingen, Germany

5 - Institute of Biology, Microbiology/Molecular Physiology of Prokaryotes, University of Freiburg, Freiburg, Germany

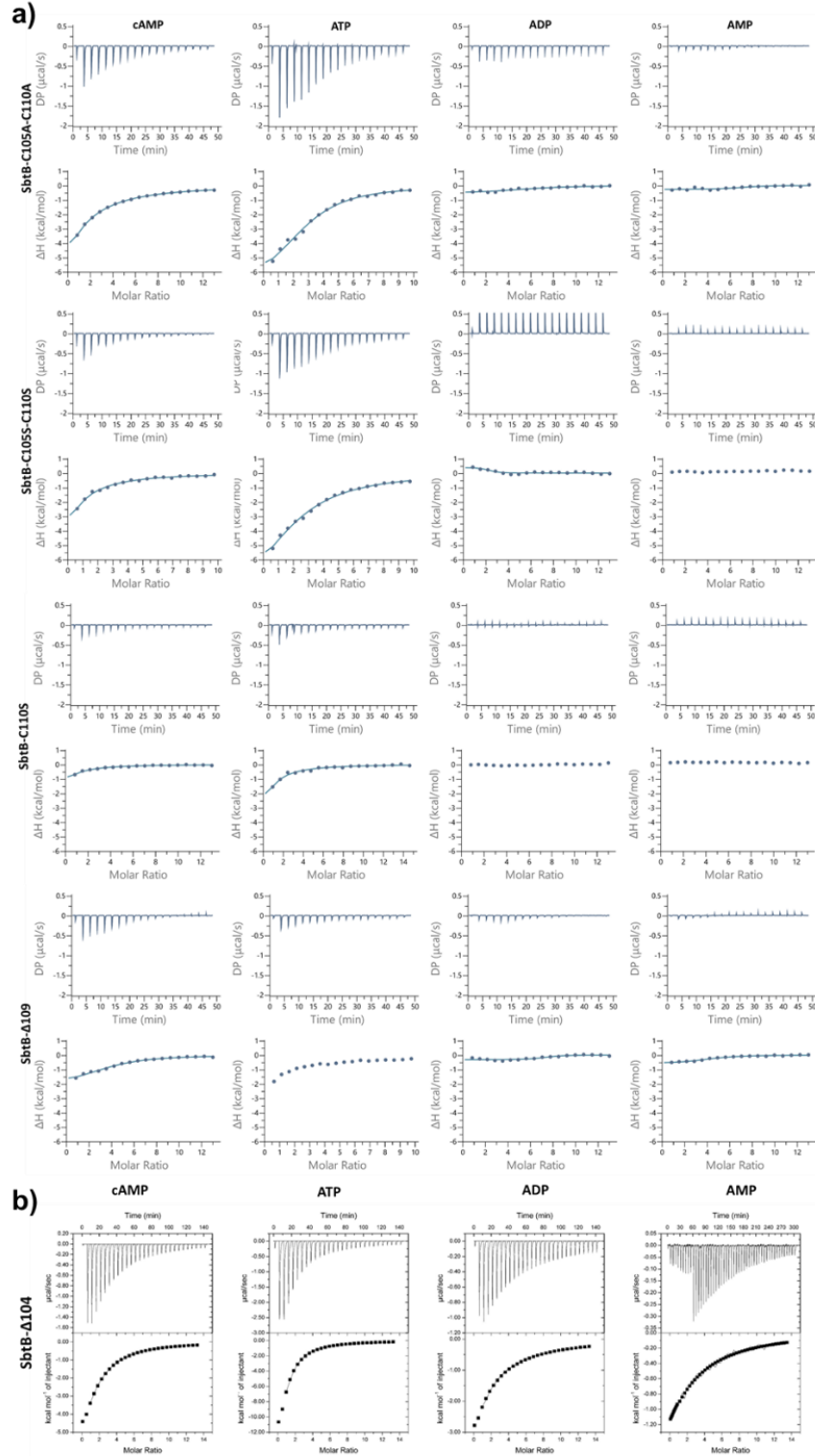
6 – Interdisciplinary Faculty, Department Life, Light and Matter, University of Rostock, Rostock, Germany

**\*Corresponding authors:** Khaled A. Selim, Microbiology/ Molecular Physiology of Prokaryotes, Institute of Biology, University of Freiburg, Schänzlestraße 1, 79104 Freiburg, Germany, Email: [khaled.selim@biologie.uni-freiburg.de](mailto:khaled.selim@biologie.uni-freiburg.de); Martin Hagemann, Institute of Biosciences, Department of Plant Physiology, University of Rostock, A.-Einstein-Str. 3, Rostock D-18059, Germany; Tel: +49(0)3814986110; Fax: +49(0)3814986112; Email: [martin.hagemann@uni-rostock.de](mailto:martin.hagemann@uni-rostock.de)

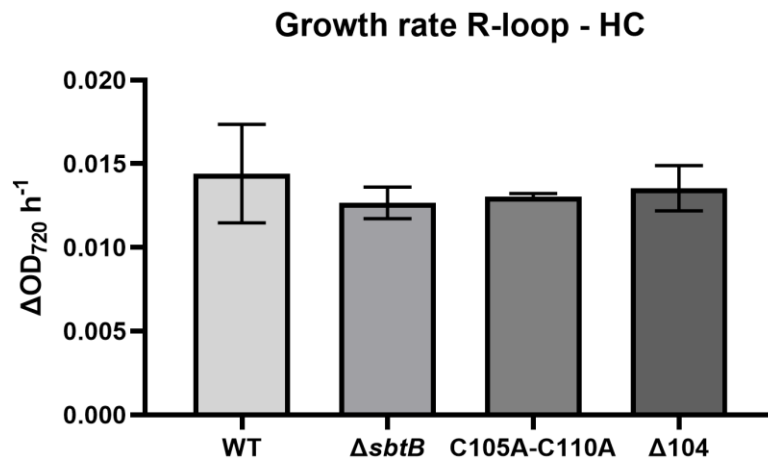
**Table S1: Primer list**

| Primers/<br>amplification   | Sequence (5'→3')  | Note/<br>Refs. |
|---|---|----------------|
| <b>Mutant generation</b>  |   |                |
| Insertion of Afel<br>restriction site<br>at the end of<br><i>sbtB</i> coding<br>sequence                  | <b>PR1_SbtB_fw:</b><br>GGATACACGGTAATGAATACC  | [13]           |
|   | <b>PR2_SbtB_Afel-STOP_rv:</b><br>CCTCAGGTCATTAAGCGCTGAAAGTATGCCCATAAAGTACTTC                                      | [13]           |
| Mutants<br>verification   | <b>2059:</b><br>GTTATTTTGTCTGCTCAAAC  | [13]           |
|   | <b>2092:</b><br>GGATCCCCCAATAGTTTATGGTC   | [13]           |
|   | <b>#PR6_SbtB_pUC19_seq_rv:</b><br>CTGCCCTGCTGCGTAACATCGTTGC   | This<br>study  |
| <b>Recombinant proteins</b>   |   |                |
| C-terminal<br>StrepII-tagged<br>ScSbtB<br>( <i>slr1513</i> );<br>(pASK-<br>IBA3_ScSbtB-<br>strep plasmid) | 1256_Fw:<br>GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATG<br>GCTAAACCAGCGAACAAGCTCG                            | [11]           |
|   | 1257_Rv:<br>AAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTACAGCCCT<br>CAGGGCCACAGAAAAG                                  | [11]           |
| C-terminal<br>StrepII-tagged<br>ScSbtB-Δ104<br>(pASK-<br>IBA3_ScSbtB-<br>Δ104)                            | 1256_Fw:<br>GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAAACCAGCGAACAAGCTCG                                | [13]           |
|   | 1663_Rv_SbtB delta 104:<br>CAAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGAAAGTATGCCCATAAAGTACTTCTGC                   | [13]           |
| C-terminal<br>StrepII-tagged<br>ScSbtB-Δ109<br>(pASK-<br>IBA3_ScSbtB-<br>Δ109)                            | 1256: Slr1513_Fw:<br>GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAAACCAGCGAACAAGCTCG                       | This<br>study  |
|   | 1661: Rv_SbtB W/o C 110:<br>AAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGCCCTCAGGGCCACAGAAAAG                         | This<br>study  |
| C-terminal<br>StrepII-tagged<br>ScSbtB-<br>C105S+C110S<br>(pASK-<br>IBA3_ScSbtB-<br>C105S+C110S)          | 1256_Fw:<br>GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATG<br>GCTAAACCAGCGAACAAGCTCG                            | [13]           |
|   | 1761_Rv_SbtB-C105+110S<br>CAAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGCTGCCCTCAGGGCCGCTGAAAGTATGCCCATAAAGTACTTCTGC  | [13]           |
| C-terminal<br>StrepII-tagged<br>ScSbtB-<br>C105A+C110A<br>(pASK-<br>IBA3_ScSbtB-<br>C105A+C110A)          | 1256_Fw:<br>GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATG<br>GCTAAACCAGCGAACAAGCTCG                            | [13]           |
|   | 1759_Rv_SbtB-C105+110A<br>CAAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGCGCCCTCAGGGCCGCTGCGAAAGTATGCCCATAAAGTACTTCTGC | [13]           |
| N-terminal<br>StrepII-tagged<br>ScSbtB-WT<br>(pASK-<br>IBA5_ScSbtB-<br>WT)                                | 1530: FW: _SbtB-Native- N ST:<br>GAGCCACCCGAGTTCGAAAAAGGCGCCGACGACGACGACAGAAGTGGCTAAACCAGCGAACAAGCTC              | This<br>study  |
|   | 1531: Rv_SbtB-Native_N ST:<br>CGACCTCGAGGGATCCCCGGGTACCGAGCTCGAATTCGGGACCTTAACAGCCCTCAGGGCCACAG                   | This<br>study  |
| C-terminal<br>StrepII-tagged<br>ScSbtB-C105S<br>(pASK-<br>IBA3_ScSbtB-<br>C105S)                          | 1256: Slr1513_Fw:<br>GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAAACCAGCGAACAAGCTCG                       | This<br>study  |
|   | 1760: RV_SbtB C-105-S:<br>CAAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTACAGCCCTCAGGGCCGCTGAAAGTATGCCCATAAAGTACTTCTGC  | This<br>study  |
| C-terminal<br>StrepII-tagged<br>ScSbtB-C110S<br>(pASK-<br>IBA3_ScSbtB-<br>C110S)                          | 1256: Slr1513_Fw:<br>GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAAACCAGCGAACAAGCTCG                       | This<br>study  |
|   | 1662: Rv_SbtB-C110S:<br>AAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGCTGCCCTCAGGGCCACAGAAAAG                          | This<br>study  |

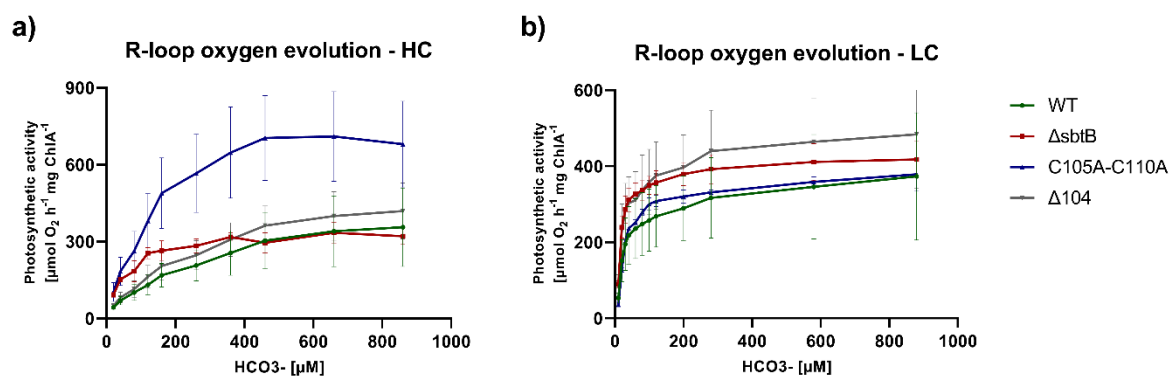
|  |   |      |
|--|---|------|
| N-terminal His <sub>6</sub> -tagged TrxA (slr0623); (pET15b_TrxA-His <sub>6</sub> plasmid) | pET15b_slr0623_fw:<br>CAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGCTCGAGATGAGTGCTACCCCTCAAGTTTC     | [13] |
|  | pET15b_slr0623_rev:<br>CCCTCAAGACCCGTTTAGAGGCCCAAGGGTTATGCTAGTTATTGCTCAGCGGTGGCAGCAGCCAAC | [13] |



**Figure S1: Isothermal titration calorimetry (ITC) analysis of ligand binding properties of the SbtB R-loop variants.** a) Upper panels show the raw ITC data in the form of the heat produced during the titration of wild-type and site-specific variant SbtB (trimeric concentration), respectively, with different effector molecules; lower panels show the binding isotherms and the best-fit curves according to the one binding site models for trimeric SbtB. b) ITC analysis of ligand binding properties of the SbtB R-loop variant ( $\Delta 104$ ).



**Figure S2: Growth rate analysis of *Synechocystis* R-loop mutants under HC conditions.** Growth of strains wild type (WT),  $\Delta sbtB$ , C105A-C110A, and  $\Delta 104$  was analyzed at high CO<sub>2</sub> (5%, HC) and continuous light in the Multicultivator MD1000. The growth rate ( $\Delta OD_{720}/h$ ) was determined during the exponential phase.



**Figure S3: Bicarbonate-dependent oxygen evolution analysis of *Synechocystis* R-loop mutants.**

Bicarbonate-dependent oxygen production via photosynthesis was measured for strains wild type (WT), ΔsbtB, C105A-C110A, and Δ104 that were acclimated to either high CO<sub>2</sub> (5%, HC) (a) or ambient CO<sub>2</sub> (0.04%, LC) (b) conditions.

**Sample A):** SbtB C-terminal tagged (oxidized)

Experimental Monoisotopic Mass: 13063.55

N A K P A N K L V I V T E K I L L K K I A K I I D E 25  
26 S G A K G Y T V M N T G G K G S R N V R S S G Q P 50  
51 N T S D I E A N I K F E I L T E T R E M A E E I A 75  
76 D R V A V K Y F N D Y A G I I Y I C S A E V L Y G 100  
101 H T F C G P E G C S A W S H P Q F E K C

S-S

**Sample B.1):** SbtB C-terminal tagged (DTT reduced)

Experimental Monoisotopic Mass: 13063.57

N A K P A N K L V I V T E K I L L K K I A K I I D E 25  
26 S G A K G Y T V M N T G G K G S R N V R S S G Q P 50  
51 N T S D I E A N I K F E I L T E T R E M A E E I A 75  
76 D R V A V K Y F N D Y A G I I Y I C S A E V L Y G 100  
101 H T F C G P E G C S A W S H P Q F E K C

S-S

**Sample B.2):** SbtB C-terminal tagged (DTT reduced)

Experimental Monoisotopic Mass: 13065.56

N A K P A N K L V I V T E K I L L K K I A K I I D E 25  
26 S G A K G Y T V M N T G G K G S R N V R S S G Q P 50  
51 N T S D I E A N I K F E I L T E T R E M A E E I A 75  
76 D R V A V K Y F N D Y A G I I Y I C S A E V L Y G 100  
101 H T F C G P E G C S A W S H P Q F E K C

SH SH

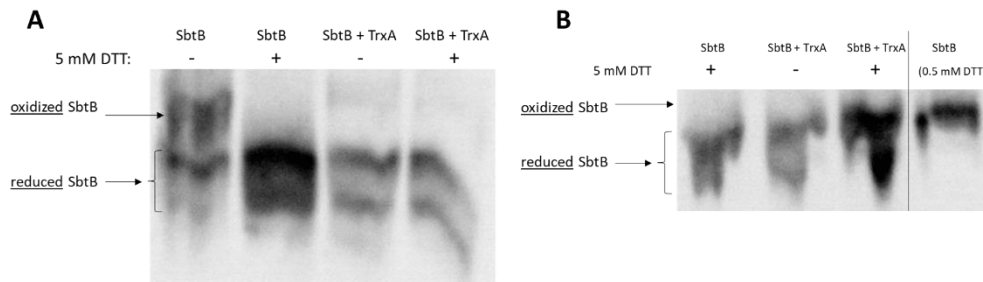
**Sample C):** SbtB C-terminal tagged (DTT-TrxA-reduced)

Experimental Monoisotopic Mass: 13065.59

N A K P A N K L V I V T E K I L L K K I A K I I D E 25  
26 S G A K G Y T V M N T G G K G S R N V R S S G Q P 50  
51 N T S D I E A N I K F E I L T E T R E M A E E I A 75  
76 D R V A V K Y F N D Y A G I I Y I C S A E V L Y G 100  
101 H T F C G P E G C S A W S H P Q F E K C

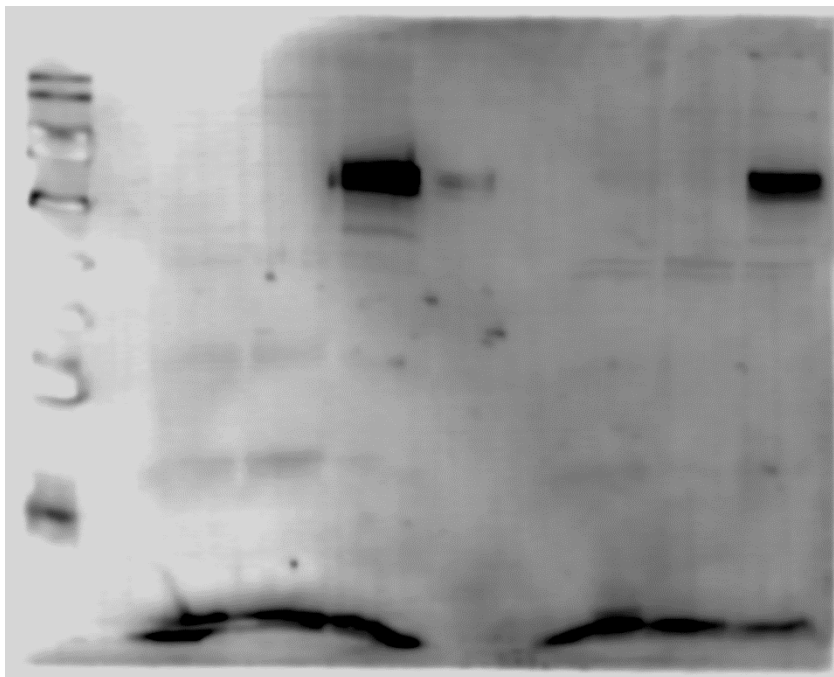
SH SH

**Figure S4: Peptide sequence of oxidized and reduced SbtB.** Peptide fragment maps were generated with the MS/MS data shown in Fig. 5. **A-C** indicate the presence of a disulfide bond in (**Sample A**; black peaks in Figure 5A) and the absence of such bond in (**Sample C**; red peak in Figure 5 C). **Sample B** contained SbtB in both oxidized and reduced states.

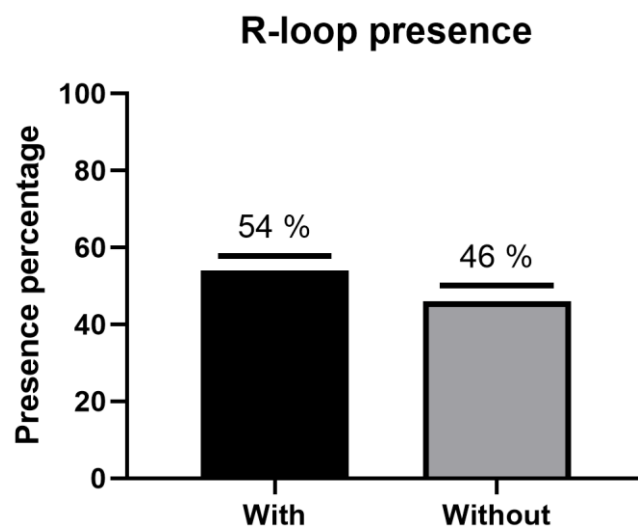


**Figure S5: Effect of TrxA and/or DTT additions on reduction of SbtB, resolved on Urea-PAGE and detected using SbtB antibody.** Urea-PAGE gel showing the reduction of SbtB by 5 mM DTT or equimolar TrxA concentrations (**A**), compared to 0.5 mM DTT (**B**). The full-size Western-blot is shown in Suppl. Fig. S8.

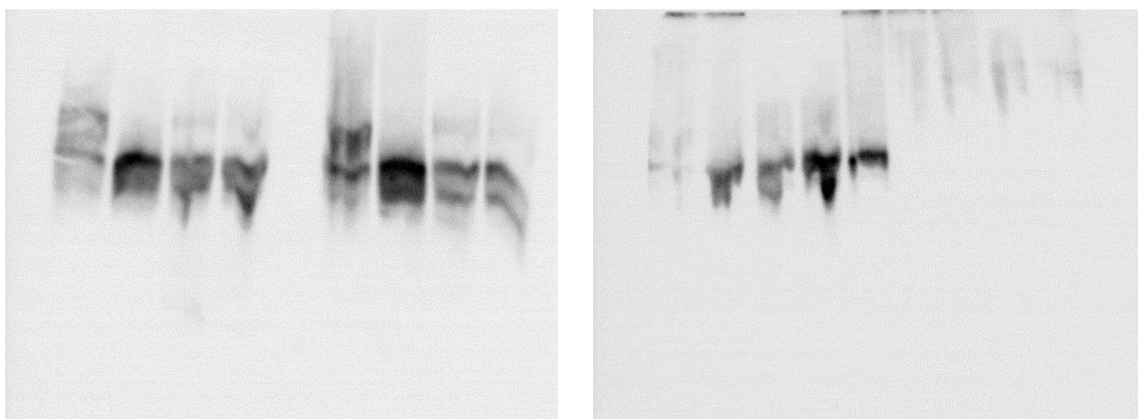




**Figure S6: Full-size Western blot of figure 6a.** Please note that the high molecular mass signals most probably represent stable TrxA/SbtB complexes.



**Figure S7: Presence of the R-loop with the highly conserved cysteine residues among cyanobacterial SbtB homologs.** R-loop presence percentage was calculated from a multiple sequence alignment of 460 SbtB sequences (sequences obtained from the NCBI data base in June 2023, alignment done with ClustalW).



**Figure S8: Full-size Western blots of Suppl. figure S5.**