Supplementary Information

RNA recognition of the HrpB bacterial DExH-box helicase is mediated by its additional domains

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Inventory of Supplementary information

Supplementary Figure 1. Sequence conservation among bacterial HrpB proteins.

Supplementary Figure 2. Sequence alignment of characterized DExH-box helicases.

Supplementary Figure 3. His_{10}Smt3 cleavage, gel filtration and ATPase activity.

Supplementary Figure 4. Deoxyribonucleotide specificity.

Supplementary Figure 5. HrpB ATPase metal, pH and salt dependence.

Supplementary Figure 6. Glycerol gradient sedimentation of HrpB.

Supplementary Figure 7. Workflow of the HDX-MS experiments.

Supplementary Figure 8. Peptide map of HrpB showing peptides that were analysed by HDX-MS.

Supplementary Figure 9. Deuterium uptake plots (% deuteration ± standard deviation) over time of flaking regions represented in Figure 5.

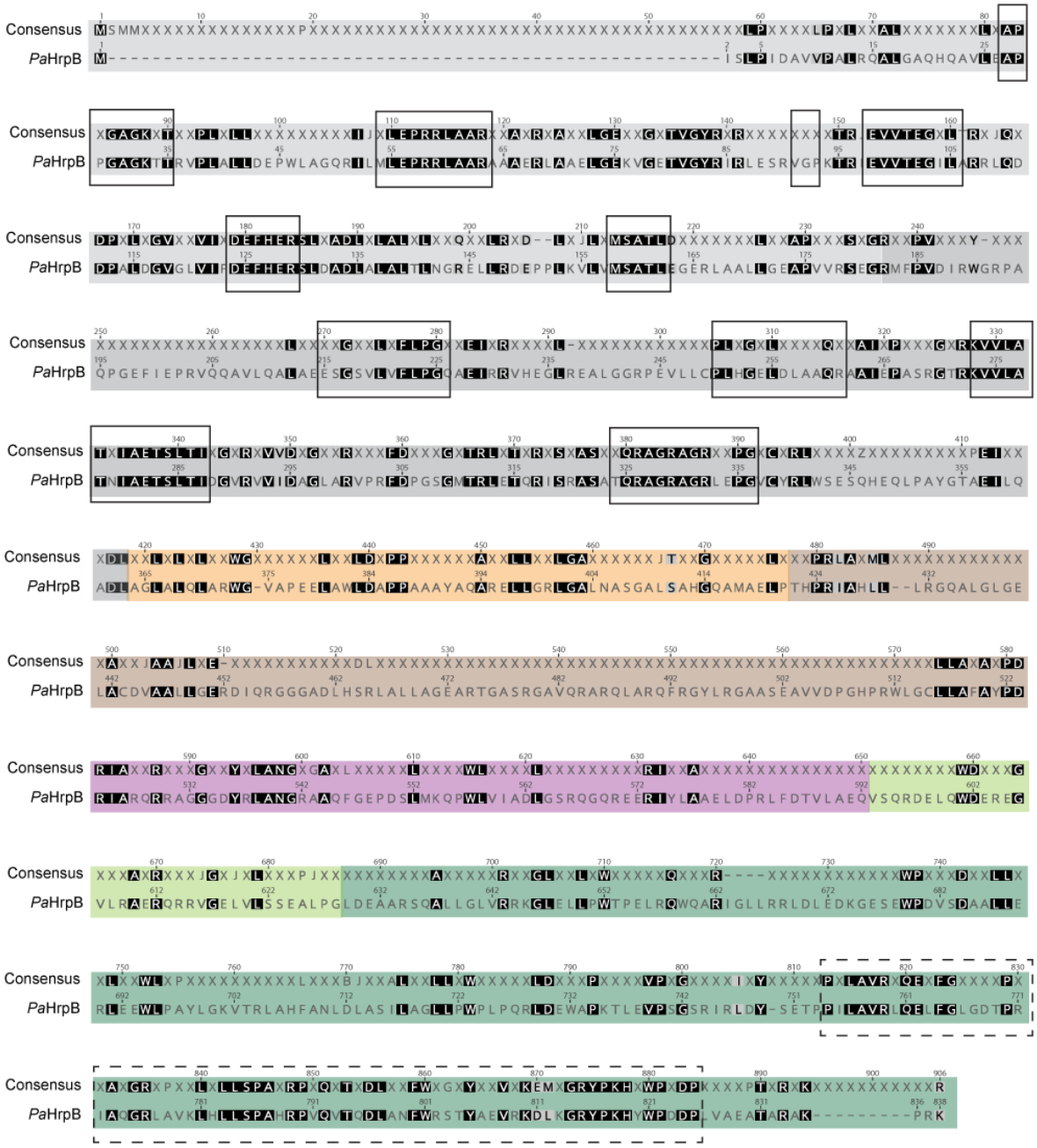
Supplementary Figure 10. *Pa*HrpB model.

Table S1. Strains and plasmids used in this study.

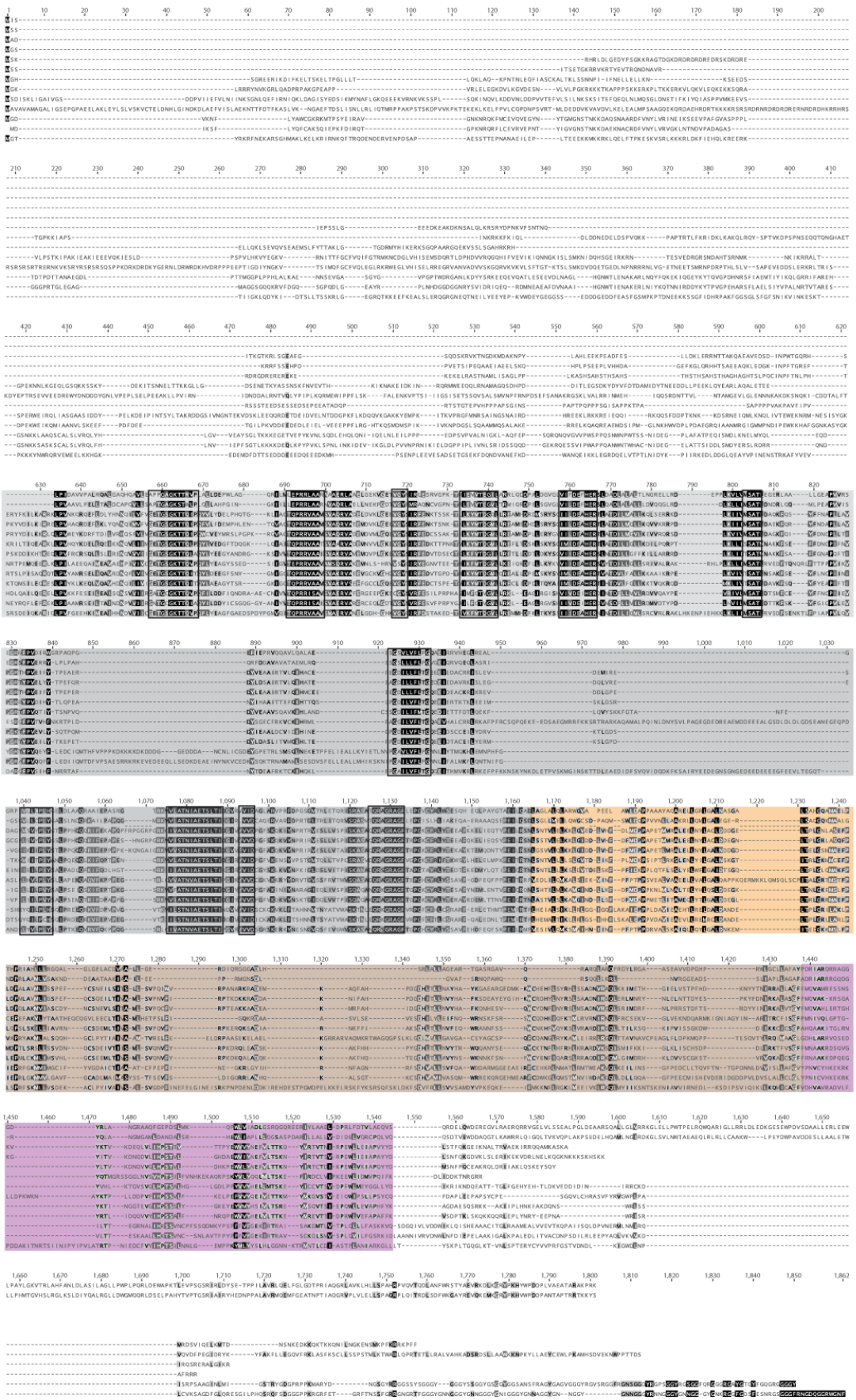
Table S2. List of primers used in this study.

Table S3. HDX-MS experimental details.

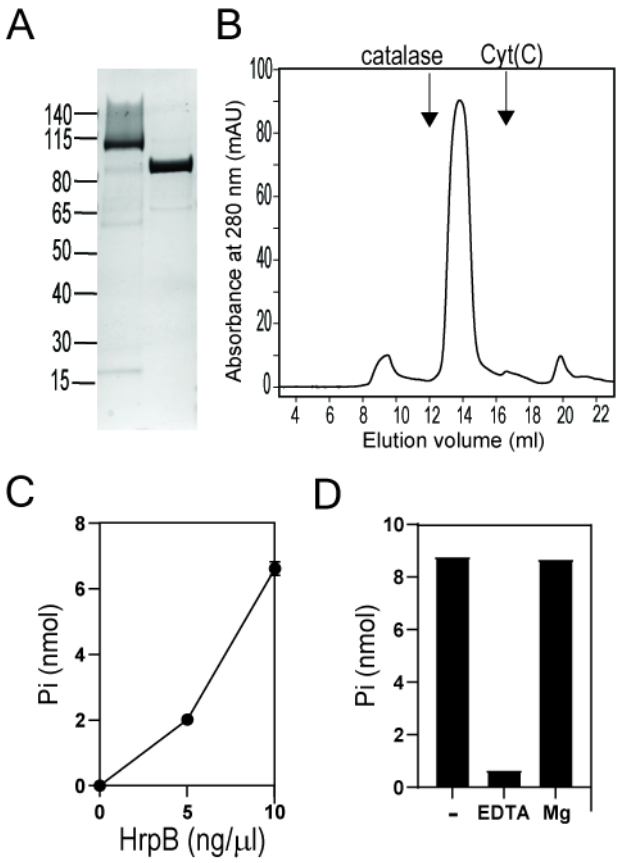
Table S4: HDX-MS data (see Excel file)**.**



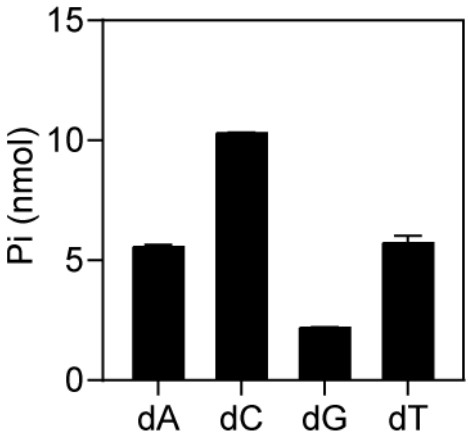
Supplementary Figure 1. Sequence conservation among bacterial HrpB proteins. Alignment of *P. aeruginosa* HrpB and bacterial HrpB sequence consensus. The consensus was created by MAFFT alignment of 700 HrpB homologous proteins retrieved from the October 2017 full-genomes NCBI database. The consensus threshold was set to 65% and it displays only bases matching at least 65% of all the sequences. Sequence region corresponding to the RecA-like, WH, HB, and OB domains are coloured as in Figure 1, while boxes highlight stretch of conserved motifs.


1. PaHrpB
2. EcHrpB
3. CfPrp43
4. ScPrp43
5. HsPrp43
6. ScPrp2
7. ScPrp16
8. MmDHX37
9. ScPrp22
10. HsDHX8
11. HsDHX9
12. DmMLE
13. ScDhr1

35 **Supplementary Figure 2. Sequence alignment of characterized DExH-box helicases.**
36 Strictly conserved residues are in white on a black background while partially conserved
37 residues are boxed on a grey background. Sequence region corresponding to the RecA-like,
38 WH, HB, and OB domains are highlighted by boxes coloured as in Figure 1. Pa, *P. aeruginosa*;
39 Ec, *E. coli*, Ct, *Chaetomium thermophilum*; Hs, *Homo sapiens*; Sc, *Saccharomyces cerevisiae*;
40 Mm, *Mus musculus*; Dm, *Drosophila melanogaster*.



Supplementary Figure 3. His_{10}Smt3 cleavage, gel filtration and ATPase activity. (A) 1 mg of His_{10}Smt3-HrpB was digested with 12.5 µg of ULP1 (home-made Smt3-specific protease) in buffer A (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 10% glycerol) for 2 hrs at 4°C. Coomassie stained SDS page gel of the undigested and digested HrpB (1 µg) is shown. The positions and sizes (in kDa) of marker proteins are indicated on the left. (B) 1 mg of digested HrpB was purified further through a Superdex-200 (S200; Akta) column equilibrated in Buffer A containing 1mM EDTA. Size-exclusion chromatography elution profile (ml) is shown. (C) ATPase activity of S200-purified untagged HrpB. Reaction mixtures (15 µl) containing 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 2 mM $MgCl_2$, 1 mM [γ-^{32}P] ATP, 250 ng/µl Poly(A) and untagged HrpB were incubated for 15 min at 37 °C. Pi release was plotted as a function of input protein. Data are the average ± SEMs from three independent experiments. (D) Metal dependence. Reaction mixtures (15 µl) containing 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 1 mM [γ-^{32}P] ATP, 250 ng/µl Poly(A), 10 ng/µl S200-purified untagged HrpB, and either No divalent cation (-), 2 mM of EDTA or 2 mM of $MgCl_2$ were incubated for 15 min at 37 °C. The extend of ATP hydrolysis is plotted.



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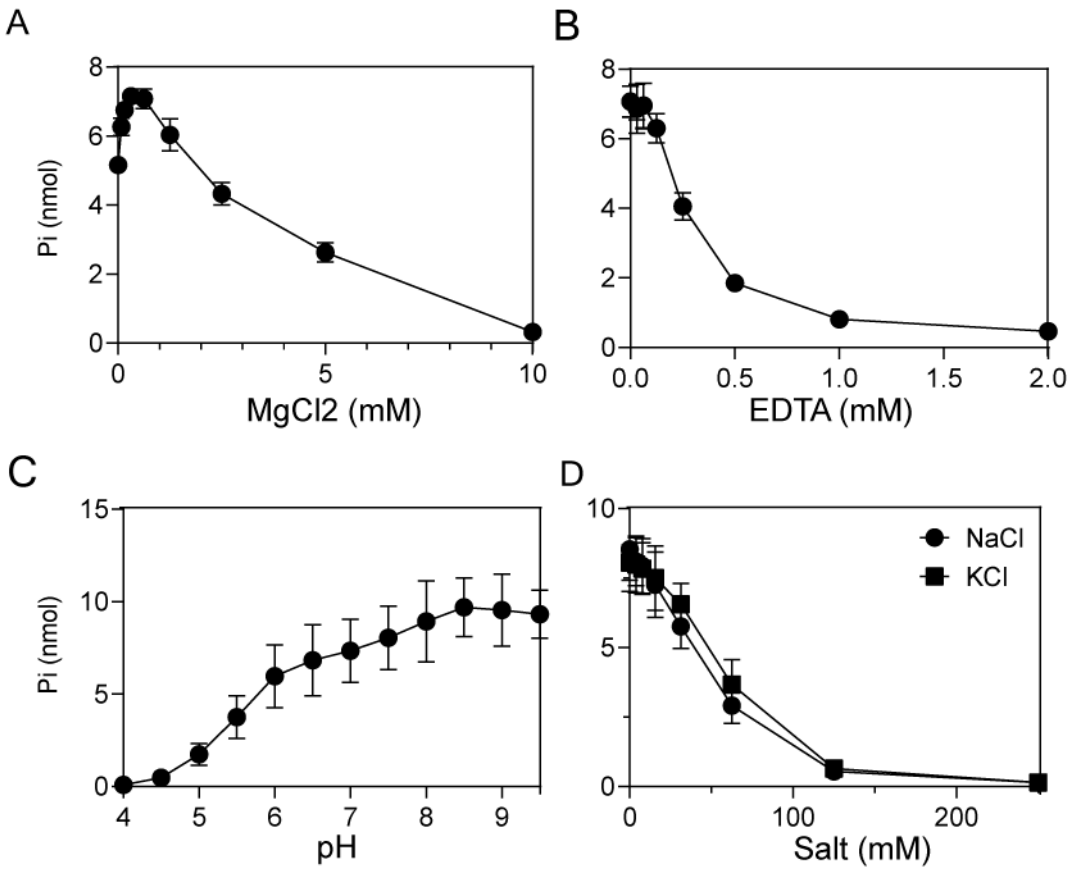
58 **Supplementary Figure 4. Deoxyribonucleotide specificity.** Reaction mixtures (15 µl)
59 containing 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 2 mM $MgCl_2$, 250 ng/µl Poly(A), 5 ng/µl WT
60 HrpB (or no added enzyme) and 1 mM deoxyribonucleotide triphosphate as specified were
61 incubated for 15 min at 37 °C. The reactions were quenched by adding 1 ml of malachite green
62 reagent. Phosphate release was determined by measuring A620 and extrapolating the value
63 to a phosphate standard curve. Data are the average ± SEMs from three independent
64 experiments.



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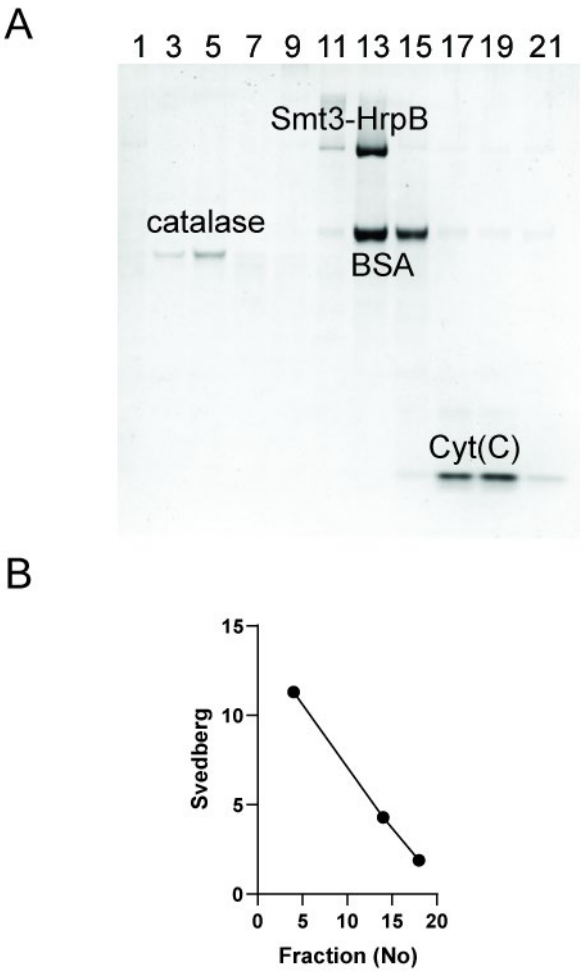
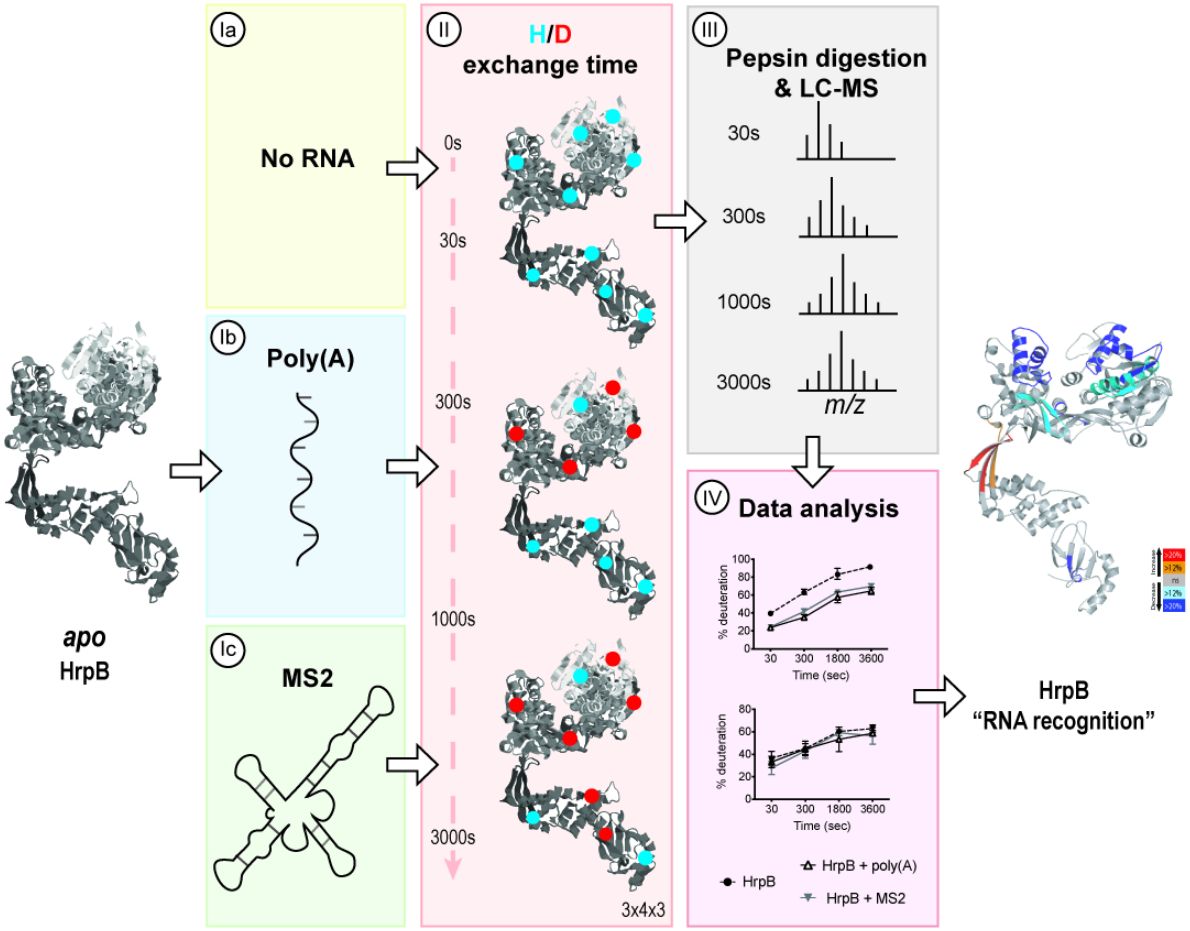
66 **Supplementary Figure 5. HrpB ATPase metal, pH and salt dependence**. (A) Magnesium
67 titration. Reaction mixtures (15 µl) containing 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 1 mM [γ-
68 ^{32}P] ATP, 250 ng/µl Poly(A), 10 ng/µl WT HrpB, and $MgCl_2$ as specified were incubated for 15
69 min at 37 °C. Pi release was plotted as a function of magnesium concentration. (B) EDTA
70 titration. Reaction mixtures (15 µl) containing 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 2 mM
71 $MgCl_2$, 250 ng/µl Poly(A), 10 ng/µl WT HrpB and EDTA as specified were incubated for 15 min
72 at 37 °C. Pi release was plotted as a function of EDTA concentration. (C) pH dependence.
73 Reaction mixtures containing either 50 mM Tris acetate (pH 5.0 to 7.0), or Tris-HCl (pH 7.5 to
74 9.5), 1 mM DTT, 2 mM MgCl2, 1 mM [γ-^{32}P] ATP, 250 ng/µl Poly(A), and 10 ng/µl WT HrpB,
75 were incubated for 15 min at 37 °C. Pi release was plotted as a function of pH. (D) Inhibition
76 of HrpB ATPase by salt. Reaction mixtures (15 µl) containing 50 mM Tris-HCl (pH 8.0), 1 mM
77 DTT, 2 mM $MgCl_2$, 250 ng/µl Poly(A), 10 ng/µl WT HrpB, and either NaCl or KCl as specified
78 were incubated for 15 min at 37 °C. Pi release was plotted as a function of salt concentration.
79 Data are the average ± SEMs from three independent experiments.

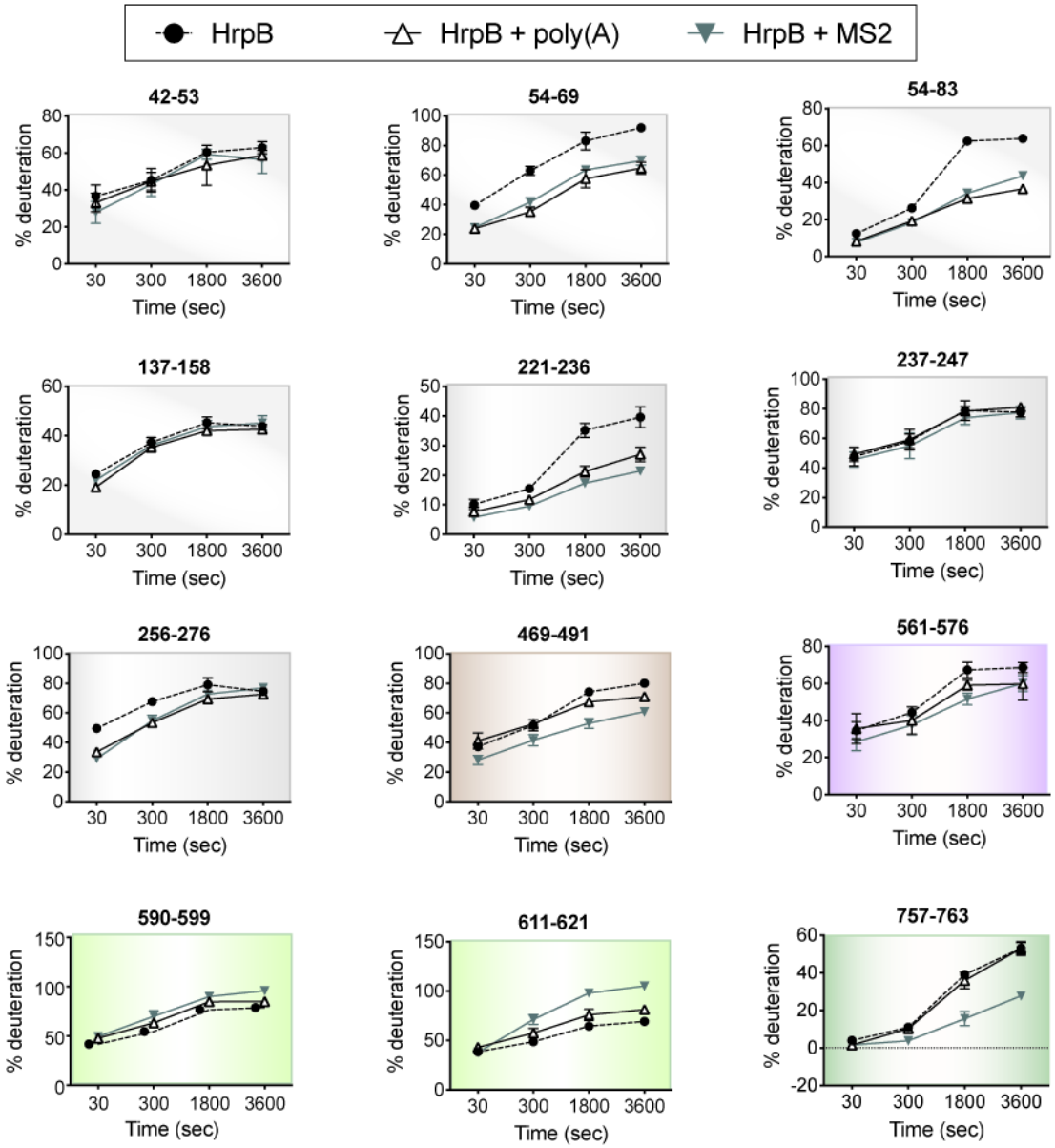


Supplementary Figure 6. Glycerol gradient sedimentation of HrpB. His_{10}Smt3-HrpB was sedimented in a glycerol gradient. Briefly, an aliquot (50 µg) of the nickel-agarose preparation of His_{10}Smt3-HrpB was mixed with catalase (40 µg), BSA (50 µg), and cytochrome c (50 µg). The mixture was applied to a 4.8-ml 15–30% glycerol gradient containing 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 2 mM DTT, and 0.05 % Triton-X100. The gradient was centrifuged in a SW50 rotor at 48,000 rpm for 19 h at 4 °C. Fractions (0.2 ml) were collected from the bottom of the tube. Aliquots (20 µl) of odd-numbered gradient fractions were analyzed by SDS-PAGE. The Coomassie Blue-stained gel is shown in panel A. (B) A plot of the S (Svedberg) values of the three standards versus fraction number is shown. This graphic allowed us to calculate an S value of 5.16 for His_{10}Smt3-HrpB.

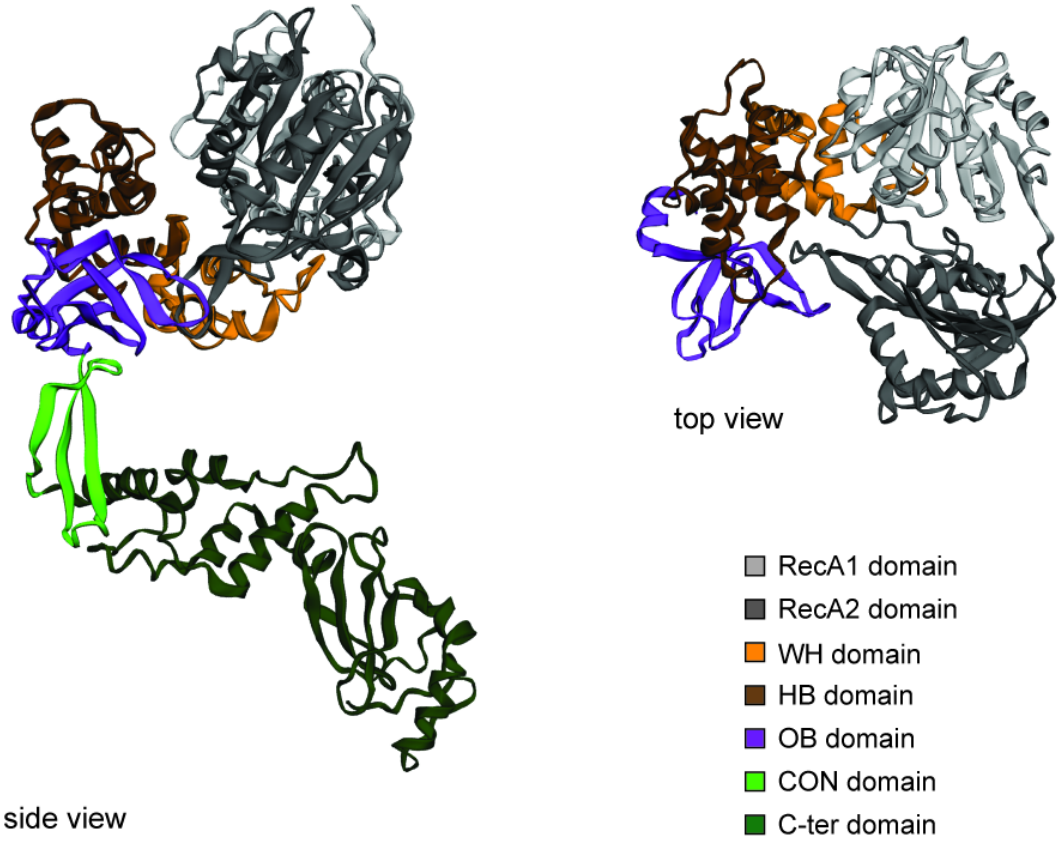


Supplementary Figure 7. Workflow of the HDX-MS experiments. HDX profile of HrpB was studied on three different conditions, e.g. *apo* protein (Ia), protein + poly(A) (Ib), and protein + MS2 RNA (Ic). For every condition, the sample was incubated with deuterated solvent and the reaction was terminated (pH 2.4, 0°C) after 30, 300, 1000 and 3000 seconds (II). Samples were digested under quenching conditions by pepsin and peptides were separated by liquid chromatography followed by mass spectrometry to identify and characterized deuterium-incorporated peptides (III). Every condition has been performed in triplicates (3 conditions x 4 timepoints x 3 replicates). Finally, data analysis included statistical tests and graphical representation of the data (see Figure 5).

Supplementary Figure 8. Peptide map of HrpB showing peptides that were analysed by HDX-MS. Coverage was 96 %. Note that the ORF of HrpB start at Ile133



Supplementary Figure 9. Deuterium uptake plots (% deuteration ± standard deviation) over time of flaking regions represented in Figure 5. Residue number is indicated on top of each graph. Graph background is coloured according to the domain to which they belong, according to Figure 1.



Supplementary Figure 10. *Pa*HrpB model. Side and top view of *P. aeruginosa* HrpB structure modelled based on the *E. coli* HrpB structure (PDB code: 6EUD and 6HEG). Sequence region corresponding to the RecA-like, WH, HB, and OB domains are highlighted by boxes coloured as in Figure 1.

115 **Table S1. Strains and plasmids used in this study.**

Strains	Genotype/relevant characteristics	Source
E.coli		
Rosetta (DE3)	*F- ompT hsdSB(rB- mB-) gal dcm* (DE3) pRARE (Cm^R)	Novagen
DH5α	*recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1* Δ(*lacZYA-argF*)*U169* [Φ80d*lacZ*M*15*]F^- Nal^r	(1)
HB101	*proA2 hsdS20*(r_{B^-} m_{B^-}) *recA13 ara-14 lacYI galK2 rpsL20 supE44 xyl-5 mtl-1* F^-	(1)
P. aeruginosa		
PAO1	Wild-type	(2)
PAO1Δ*hrpB*	PAO1 with a *hrpB* (PA3961) deletion	This study
Plasmids		
pET28b-10xHis-Smt3	Broad host range vector for expression of N-terminal 10xHis-Smt3-tag proteins, Kan^R	(3) and lab collection
pME3087	Suicide vector for allelic replacement; Tc^r; ColE1 replicon	(4)
pME3087_Δ*hrpB*	Suicide construct for the deletion of *hrpB* (aa 50 to 943)	This study
pSmt3_HrpB	Vector for expression of 10xHis-Smt3-HrpB	This study
pSmt3_$HrpB_{K33A}$	Vector for expression of 10xHis-Smt3-$HrpB_{K33A}$	This study
pSmt3_$HrpB_{1-365}$	Vector for expression of 10xHis-Smt3-$HrpB_{1-365}$	This study
pSmt3_$HrpB_{K33A,1-365}$	Vector for expression of 10xHis-Smt3-$HrpB_{K33A,1-365}$	This study
pSmt3_$HrpB_{1-628}$	Vector for expression of 10xHis-Smt3-$HrpB_{1-628}$	This study
pSmt3_$HrpB_{1-702}$	Vector for expression of 10xHis-Smt3-$HrpB_{1-702}$	This study
pSmt3_$HrpB_{1-390}$	Vector for expression of 10xHis-Smt3-$HrpB_{1-390}$	This study
pSmt3_$HrpB_{1-500}$	Vector for expression of 10xHis-Smt3-$HrpB_{1-500}$	This study
pSmt3_$HrpB_{1-539}$	Vector for expression of 10xHis-Smt3-$HrpB_{1-539}$	This study
pSmt3_$HrpB_{1-589}$	Vector for expression of 10xHis-Smt3-$HrpB_{1-589}$	This study
pRK2013	Helper plasmid; Tra^+ Km^r	(1)

116

117 **Table S2. List of primers used in this study.**

Name	Sequence[a] (5' → 3')	Restriction site[#]
pHrpB-Smt3.1	CCAAGCTTCAATTTCCCTACCCATCGACG	HindIII
pHrpB.rev	CGCTCGAGCTACTTGCGTGGCTTGGCCCGG	XhoI
pHrpBK33A.fw	CCGGGTGCCGGCGCGACCACCCGGGTG	
pHrpBK33A.rev	CACCCGGGTGGTCGCGCCGGCACCCGG	
pHrpBA390STOP.fw	CCCGCGGCGGCCTAGGCCCAGGCCCGCGAG	
pHrpBA390STOP.rev	CTCGCGGGCCTGGGCCTAGGCCGCCGCGGG	
pHrpBV702STOP.fw	CTATCTCGGCAAGGTCTAGCGCCTGGCTCACTTC	
pHrpBV702STOP.rev	GAAGTGAGCCAGGCGCTAGACCTTGCCGAGATAG	
pHrpBA500Stop.fw	CTGCGCGGCGCCGCCTAGGAGGCGGTCGTCGATC	
pHrpBA500Stop.rev	GATCGACGACCGCCTCCTAGGCGGCGCCGCGCAG	
pHrpBG365Stop.fw	GATCTGGCCGGGTAGGCCCTGCAACTG	
pHrpBG365Stop.rev	CAGTTGCAGGGCCTACCCGGCCAGATC	
pHrpBA539Stop.fw	CTACCGGCTGGCCTAGGGACGCGCTGCG	
pHrpBA539Stop.rev	CGCAGCGCGTCCCTAGGCCAGCCGGTAG	
pHrpBA589Stop.fw	GATACGGTCCTGGCGTAGCAGGTCAGCCAG	
pHrpBA589Stop.rev	CTGGCTGACCTGCTACGCCAGGACCGTATC	
pHrpBA628Stop.fw	GCGCTACCCGGCTAGGACGAAGCGGCG	
pHrpBA628Stop.rev	CGCCGCTTCGTCCTAGCCGGGTAGCGC	
pHrpB.1	GCCGGTACCGGCGAAAAGGTCGGCGAGAC	
pHrpB.2	GCCCTGCAGATGAACTCGCCGGGTTGCGC	
pHrpB.3	GCCCTGCAGGGCAGTGAAGCTGCACCTGC	
pHrpB.4	GCCAAGCTTAACGCTGGTATCGCCTCTAC	
rpoD.fw	GGGCGAAGAAGGAAATGGTC	
rpoD.rev	CAGGTGGCGTAGGTGGAGAA	

118 [#]restriction sites underlined

119 **Table S3. HDX-MS experimental details.**

Description	HrpB apo	HrpB + poly(A) RNA	HrpB + MS2 RNA
Reaction volume	50 ul	50 ul	50 ul
% D2O in the reaction	79%	79%	79%
Temperature	0°C	0°C	0°C
Time course (sec)	30, 300, 1800, 3600	30, 300, 1800, 3600	30, 300, 1800, 3600
Control samples	Non-deuterated (ND) and fully deuterated (FD) HrpB protein		
Quench buffer	3 M Gdn-HCl/ 0.1 M NaH2PO4 pH 2.5/ 1 % Formic Acid		
Quench buffer volume	20 ul	20 ul	20 ul
Number of peptides analyzed	189	189	189
Sequence coverage	96%	96%	96%
Replicates (for each incubation time)	3, 3, 3, 3	3, 3, 3, 3	3, 3, 3, 3
Standard deviation average (all time points, Nb of Deuterons)	0.14	0.16	0.15
Criteria for HDX rate difference	Difference of HDX level at a given timepoint is > 12 % and > 0.6 Da and p values of student t-test is < 0.05		

120

121 **Table S4: HDX-MS data (see Excel file).**

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123 ## Additional references

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