1 Supplementary Information

- RNA recognition of the HrpB bacterial DExH-box helicase is mediated by its additional
 domains
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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	1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 811
 CrPrp43 ScPrp43 ScPrp43 ScPrp2 ScPrp16 MmDHX37 ScPrp22 HsDHX8 HsDHX9 DmMLE ScDhr1 	Solo Solo Solo Solo Solo Solo Solo Solo Solo TST EXEMPTION TO ACCOUNT SOLONDATION OF SOLVAACTION CONDITION OF SOLVAACTION CONDITION OF SOLVAACTION CONDITION OF SOLVAACTION OF SOLVAATTION OF SOLVAACTION OF SOLVAATTION OF
1. PaHrpB 2. EcHrpB 3. CrPrp43 4. ScPrp43 5. HsPrp43 6. ScPrp16 8. MmDHX37 9. ScPrp16 10. HsDHX87 11. HsDHX9 12. DmMLE 13. ScDhr1	210 220 240 240 240 240 240 240 240 240 24
1. PaHrpB 2. EcHrpB 3. CfPrp43 4. ScPrp43 6. ScPrp43 6. ScPrp2 7. ScPrp16 8. MmDHX37 9. ScPrp216 10. HsDHX8 11. HsDHX8 11. HsDHX8 12. DmMLE 13. ScDhr1	470 410 440 470 410 490 500 510 520 510 540 510 540 510 500 500 610
1. PaHrpB 2. EcHrpB 3. CPrp43 4. ScPrp43 5. HsPrp43 6. ScPrp2 7. ScPrp16 8. MmDHX37 9. ScPrp21 10. HsDHX88 11. HsDHX9 12. DmMLE 13. ScDhr1	40 60 60 60 60 60 60 60 70<
1. PaHrpB 2. EcHrpB 3. CtPrp43 4. ScPrp43 6. ScPrp43 6. ScPrp43 6. ScPrp64 7. ScPrp16 8. MmDHX37 9. ScPrp16 8. MmDHX37 9. ScPrp22 10. HsDHX8 11. HsDHX9 12. DmMLE 13. ScDhr1	ED 20 20 20 20 20 20 20 20 20 20 20 20 20
1. PaHrpB 2. EcHrpB 3. CrPrp43 4. ScPrp43 6. ScPrp2 7. ScPrp2 10. HsDHX37 9. ScPrp22 10. HsDHX8 11. HsDHX9 12. DmMLE 13. ScDhr1	
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1. PaHrpB 2. EcHrpB 3. CfPrp43 4. ScPrp43 6. ScPrp43 6. ScPrp2 7. ScPrp26 8. MmDHX37 9. ScPrp26 10. HsDHX8 11. HsDHX8 11. HsDHX9 12. DmMLE 13. ScDhr1	1.460 1.470 1.480 1.490 1.590 1.690 1.600 <td< th=""></td<>
1. PaHrpB 2. EcHrpB 3. C/Prp43 4. ScPrp43 5. HsPrp43 6. ScPrp2 7. ScPrp16 8. MmDHX37 9. ScPrp22 10. HsDHX37 11. HsDHX3 11. HsDHX9 12. DmMLE 13. ScDhr1	1.460 1.670 1.680 1.700 1.700 1.700 1.700 1.700 1.700 1.700 1.700 1.700 1.700 1.700 1.700 1.600 1.610

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,
- 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. His₁₀Smt3 cleavage, gel filtration and ATPase activity. (A) 1 mg 42 of His₁₀Smt3-HrpB was digested with 12.5 µg of ULP1 (home-made Smt3-specific protease) 43 in buffer A (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 10% glycerol) for 2 hrs at 4°C. Coomassie 44 stained SDS page gel of the undigested and digested HrpB (1 µg) is shown. The positions 45 46 and sizes (in kDa) of marker proteins are indicated on the left. (B) 1 mg of digested HrpB was 47 purified further through a Superdex-200 (S200; Akta) column equilibrated in Buffer A 48 containing 1mM EDTA. Size-exclusion chromatography elution profile (ml) is shown. (C) 49 ATPase activity of S200-purified untagged HrpB. Reaction mixtures (15 µl) containing 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 2 mM MgCl₂, 1 mM [γ-³²P] ATP, 250 ng/μl Poly(A) and untagged 50 HrpB were incubated for 15 min at 37 °C. Pi release was plotted as a function of input protein. 51 Data are the average ± SEMs from three independent experiments. (D) Metal dependence. 52 Reaction mixtures (15 μ l) containing 50 mM Tris-HCl (pH 8.0), 1 mM DTT 1 mM [γ -³²P] ATP, 53 54 250 ng/µl Poly(A), 10 ng/µl S200-purified untagged HrpB, and either No divalent cation (-), 2 mM of EDTA or 2 mM of MgCl₂ were incubated for 15 min at 37 °C. The extend of ATP 55 hydrolysis is plotted. 56



Supplementary Figure 4. Deoxyribonucleotide specificity. Reaction mixtures (15 μl) containing 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 2 mM MgCl₂, 250 ng/μl Poly(A), 5 ng/μl WT HrpB (or no added enzyme) and 1 mM deoxyribonucleotide triphosphate as specified were incubated for 15 min at 37 °C. The reactions were quenched by adding 1 ml of malachite green reagent. Phosphate release was determined by measuring A620 and extrapolating the value to a phosphate standard curve. Data are the average ± SEMs from three independent experiments.



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



81 **Supplementary Figure 6. Glycerol gradient sedimentation of HrpB.** His₁₀Smt3-HrpB was 82 sedimented in a glycerol gradient. Briefly, an aliquot (50 µg) of the nickel-agarose preparation of His₁₀Smt3-HrpB was mixed with catalase (40 µg), BSA (50 µg), and cytochrome c (50 µg). 83 The mixture was applied to a 4.8-ml 15–30% glycerol gradient containing 50 mM Tris-HCI (pH 84 8.0), 100 mM NaCl, 1 mM EDTA, 2 mM DTT, and 0.05 % Triton-X100. The gradient was 85 centrifuged in a SW50 rotor at 48,000 rpm for 19 h at 4 °C. Fractions (0.2 ml) were collected 86 from the bottom of the tube. Aliquots (20 µl) of odd-numbered gradient fractions were analyzed 87 by SDS-PAGE. The Coomassie Blue-stained gel is shown in panel A. (B) A plot of the S 88 (Svedberg) values of the three standards versus fraction number is shown. This graphic 89 allowed us to calculate an S value of 5.16 for His₁₀Smt3-HrpB. 90



Supplementary Figure 7. Workflow of the HDX-MS experiments. HDX profile of HrpB was 92 93 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 94 reaction was terminated (pH 2.4, 0°C) after 30, 300, 1000 and 3000 seconds (II). Samples 95 were digested under quenching conditions by pepsin and peptides were separated by liquid 96 chromatography followed by mass spectrometry to identify and characterized deuterium-97 incorporated peptides (III). Every condition has been performed in triplicates (3 conditions x 4 98 timepoints x 3 replicates). Finally, data analysis included statistical tests and graphical 99 100 representation of the data (see Figure 5).

1 5	10	15	2D	25	30	35	4D	45	50	55	60	EKIKK	70	75	80	85	9D
MGHHE	ННННН	НН <i>В</i> В В	HIEGR	H M A S M	SDSEV	NQEAK	PEVKP	EVKPE	THINL	KVSDG	SSEIF		TTPLR	RLMEA	FAKRQ	GKEMD	SLRFL
91 95	100	105	110	115	120	125	130	135	140	145	150	155	160	165	170	175	180
YDGIF	I Q A D Q	TPEDL	DMEDN	DIIEA	HREQI	GGSEF	ELRRQ	ASISL	PIDAV	VPALR	g A L G A	Q H Q A V	L E A P P	GAGKT	T R V P L	A L L D E	PWLAG
181 185	190	195	200	205	210	215	220	225	TRIEV	235	240	245	250	255	260	265	270
RILM	LEPRR	LAARA	A A E R L	A A E L G	E K V G E	T V G Y R	IRLES	RVGPK		V T E G I	LARRL	D D P A	L D G V G	L V I F D	Е F H E R	SLDAD	LALAL
271 275	280	285	290	295	300	305	310	315	320	325	330	335	340	345	350	355	360
TLNGF	E L L R D	E P P L K	V L V M S	A T L E G	ERLAA	L L G E A	P V V R S	E G R M F	PVDIR	WGRPA	Q P G E F	IEPRV	Q Q A V L	Q A L A E	E S G S V	LVFLP	G Q A E I
361 365	370	375	380	385	390	395	400	405	410	415	420	425	430	435	440	445	450
RRVHE	GLREA	LGGRP	E V L L C	PLHGE	LDLAA	Q R A A I	E P A S R	G T R K V	V L A T N	I A E T S	L T I D G	V R V V I	DAGLA	RVPRF	DPGSG	MTRLE	TQRIS
451 455	46D	465	470	475	480	485	49D	495	500	505	510	515	520	525	530	535	54D
RASAT	Q R A G R	AGRLE	PGVCY	RLWSE	S Q H E Q	L P A Y G	TAEIL	Q A D L A	G L A L Q	LARWG	V A P E E	LAWLD	A P P A A	A Y A Q A	RELLG	R L G A L	NASGA
			-														
541 545	550	555	560	565	570	575	580	585	590	595	600	605	610	615	620	625	630
LSAHG	Q A M A E	L P T H P	RIAHL	L L R G Q	ALGLG	E L A C D	V A A L L	GERDI	Q R G G G	A D L H S	R L A L L	A G E A R	T G A S R	G A V Q R	A R Q L A	R Q F R G	Y L R G A
541 545 LSAHG 631 635 ASEAV	550 QAMAE 640 VDPGH	555 LPTHP 645 PRWLG	560 RIAHL 650 CLLAF	565 L L R G Q 655 A Y P D R	570 ALGLG 660 IARQR	575 E L A C D 665 R A G G G	580 V A A L L 670 D Y R L A	585 GERDI 675 NGRAA	Q R G G G Q R G G G Q F G E P	ADLHS 605 DSLMK	600 R L A L L 690 Q P W L V	605 AGEAR 695 IADLG	610 TGASR 700 SRQGQ	615 GAVQR 705 REERI	620 A R Q L A 710 Y L A A E	625 R Q F R G 715 L D P R L	630 Y L R G A 720 F D T V L
541 545 L S A H G 631 635 A S E A V 721 725 A E Q V S	550 Q A M A E 640 V D P G H 730 Q R D E L	555 LPTHP PRWLG 2WDER	560 RIAHL 650 CLLAF 740 EGVLR	L L R G Q A Y P D R A Y P D R A E R Q R	570 ALGLG IARQR RVGEL	575 E L A C D R A G G G V L S S E	560 V A A L L D Y R L A A L P G L	G E R D I 675 N G R A A D E A A R	Q R G G G Q F G E P S Q A L L	A D L H S D S L M K G L V R R	600 RLALL QPWLV KGLEL	A G E A R 695 I A D L G 1 P W T P	TGASR 700 SRQGQ ELRQW	615 GAVQR 705 REERI QARIG	A R Q L A 710 Y L A A E L L R R L	RQFRG 15 LDPRL DLEDK	630 Y L R G A 720 F D T V L G E S E W
541 545 L S A H G 631 635 A S E A V 721 725 A E Q V S 811 615	550 Q A M A B V D P G H Q R D E L A A L L B	555 L PT H P P R W L G Q W D E R Q W D E R R L E E W	560 RIAHL CLLAF F GVLR G30 LPAYL	L L R G Q A Y P D R A Y P D R A E R Q R G X V T R	570 A L G L G I A R Q R R V G E L 240 L A H F A	575 E L A C D R A G G G V L S S E 045 N L D L A	580 V A A L L O Y R L A 760 A L P G L 850 S I L A G	505 GERDI 675 DEAAA 0 EAAR 0 55 LLPWP	Q F G G G Q F G L P 770 S Q A L L 460 L P Q R L	595 A D L H S D S L M K G L V R R G L V R R 665 D E W A P	R L A L L Q P W L V K G L E L 870 K T L E V	605 AGEAR 695 IADLG 785 LPWTP PSGSR	610 T G A S R S R Q G Q F L R Q W E L R Q W G 0 I R L D Y	615 GAVQR 705 REERI QARIG QARIG SETPP	620 A R Q L A 710 Y L A A E 800 L L R L 890 I L A V R	625 R Q F R G I D P R L D I E D K L Q E L F	630 Y L R G A 720 F D T V L G E S E W 900 G L G D T

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- 102 Supplementary Figure 8. Peptide map of HrpB showing peptides that were analysed by
- 103 HDX-MS. Coverage was 96 %. Note that the ORF of HrpB start at IIe133



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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.



- 111 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).
- 113 Sequence region corresponding to the RecA-like, WH, HB, and OB domains are highlighted
- 114 by boxes coloured as in Figure 1.

Table S1. Strains and plasmids used in this study.

Strains	Genotype/relevant characteristics	Source
E.coli		
Rosetta (DE3)	<i>F- ompT hsdSB(rB- mB-) gal dcm</i> (DE3) pRARE (Cm ^R)	Novagen
DH5a	recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 Δ (lacZYA-	(1)
	argF)U169 [Ф80d/acZM15]F ⁻ Nal ^r	(')
HB101	proA2 hsdS20(r _B - m _B -) recA13 ara-14 lacYl galK2 rpsL20	(1)
_ ·	supE44 xyl-5 mtl-1 ⊦-	(-)
P. aeruginosa		(-)
PAO1	Wild-type	(2)
PAO1∆ <i>hrpB</i>	PAO1 with a <i>hrpB</i> (PA3961) deletion	This study
Plasmids		
pET28b-10xHis-	Broad host range vector for expression of N-terminal	(3) and lab
Smt3	10xHis-Smt3-tag proteins, Kan ^R	collection
pME3087	Suicide vector for allelic replacement; Tc ^r ; ColE1 replicon	(4)
pME3087_∆ <i>hrpB</i>	Suicide construct for the deletion of <i>hrpB</i> (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-HrpB1-365	This study
pSmt3_HrpBкзза,1-	Vector for expression of 10xHis-Smt3-HrpB K33A,1-365	This study
365		
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)

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

Name	Sequence ^a (5' \rightarrow 3')	Restriction site [#]
pHrpB-Smt3.1	CCAAGCTTCAATTTCCCTACCCATCGACG	HindIII
pHrpB.rev	CG <u>CTCGAG</u> CTACTTGCGTGGCTTGGCCCGG	Xhol
pHrpBK33A.fw	CCGGGTGCCGGCGCGACCACCCGGGTG	
pHrpBK33A.rev	CACCCGGGTGGTCGCGCCGGCACCCGG	
pHrpBA390STOP.fw	CCCGCGGCGGCCTAGGCCCAGGCCCGCGAG	
pHrpBA390STOP.rev	CTCGCGGGCCTGGGCCTAGGCCGCCGCGGG	
pHrpBV702STOP.fw	CTATCTCGGCAAGGTCTAGCGCCTGGCTCACTT C	
pHrpBV702STOP.rev	GAAGTGAGCCAGGCGCTAGACCTTGCCGAGAT AG	
pHrpBA500Stop.fw	CTGCGCGGCGCCGCCTAGGAGGCGGTCGTCG ATC	
pHrpBA500Stop.rev	GATCGACGACCGCCTCCTAGGCGGCGCCGCG CAG	
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	GCC <u>GGTACC</u> GGCGAAAAGGTCGGCGAGAC	
pHrpB.2	GCC <u>CTGCAG</u> ATGAACTCGCCGGGTTGCGC	
pHrpB.3	GCC <u>CTGCAG</u> GGCAGTGAAGCTGCACCTGC	
pHrpB.4	GCC <u>AAGCTT</u> AACGCTGGTATCGCCTCTAC	
rpoD.fw	GGGCGAAGAAGGAAATGGTC	
rpoD.rev	CAGGTGGCGTAGGTGGAGAA	

118 [#]restriction sites underlined

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 I	۷aH2PO4 pH 2.5/ 1 %	6 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				

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

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123 Additional references

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