

1 **Supplemental Material**

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3 **Infection of endothelial cells with *Acinetobacter baumannii* reveals remodelling**  
4 **of mitochondrial protein complexes**

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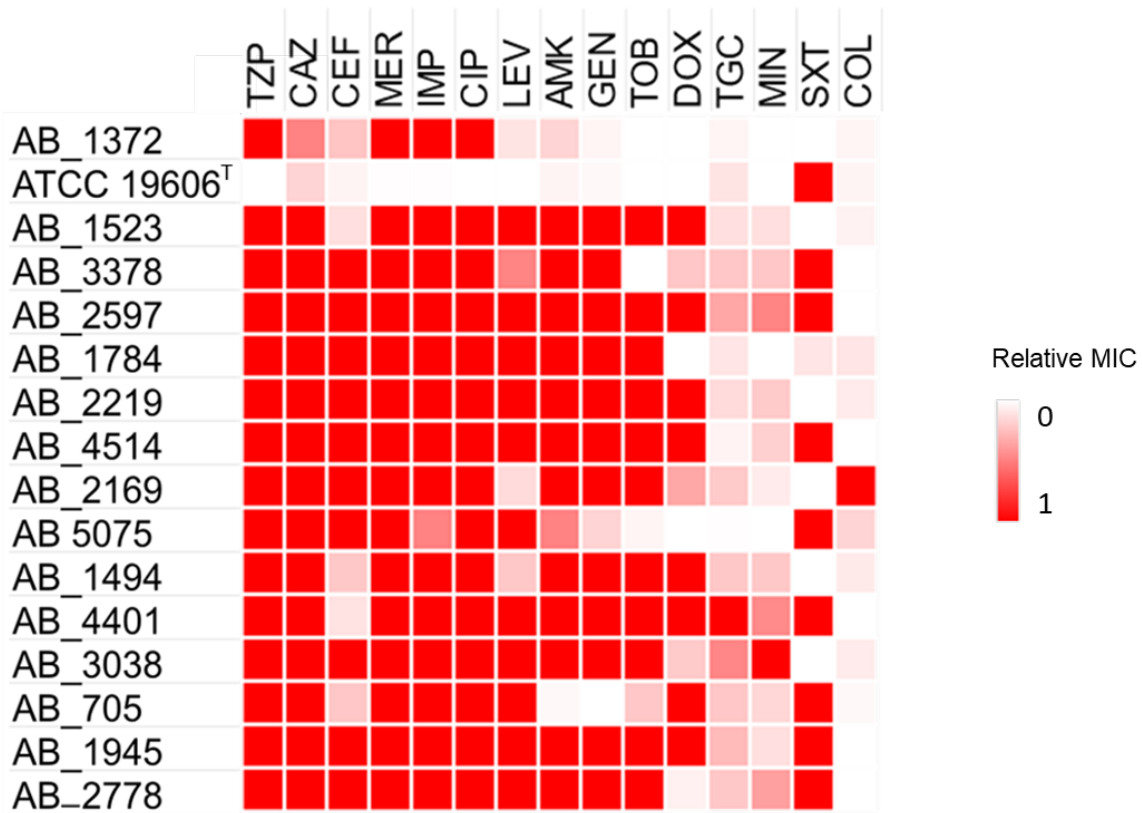
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27 **Supplementary Figures**

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31 **FIG S1** Antimicrobial susceptibility profile. The relative minimum inhibitory concentration (MIC)  
 32 indicates the ratio of the actual MIC of an isolate divided by the highest MIC value for the  
 33 respective antibiotic in the data set. The color bar displays the values. TZP,  
 34 piperacillin/tazobactam; CAZ, ceftazidime; CEF, cefepime; MER, meropenem; IMP, imipenem;  
 35 CIP, ciprofloxacin; LEV, levofloxacin; AMK, amikacin; GEN, gentamicin; TOB, tobramycin;  
 36 DOX, doxycycline; TGC, tigecycline; MIN, minocycline; SXT, trimethoprim-sulfamethoxazole;  
 37 COL, Colistin.

38

		Resistance genes																			
Isolate	ST <sup>Pas</sup>	<i>aac(3)-Ia</i>	<i>aac(6)-Iaf</i>	<i>ant(3)-Ia</i>	<i>aph(3')-Ib</i>	<i>aph(3')-Ia</i>	<i>aph(3')-Via</i>	<i>aph(6)-Id</i>	<i>armA</i>	<i>bla</i> ADC-25-like	<i>bla</i> OXA-23	<i>bla</i> OXA-66	<i>bla</i> TEM-1D	<i>catB8</i>	<i>mph(E)</i>	<i>msr(E)</i>	<i>qacE</i>	<i>sul1</i>	<i>sul2</i>	<i>tet(B)</i>	
AB_1494	2																				
AB_1523	604																				
AB_4514	2																				
AB_2169	2																				
AB_2778	2																				
AB_705	2																				
AB_1945	2																				
AB_4401	2																				
AB_1784	570																				
AB_1372	2																				
AB_3378	604																				
AB_2219	2																				
AB_2597	2																				
AB_3038	2																				

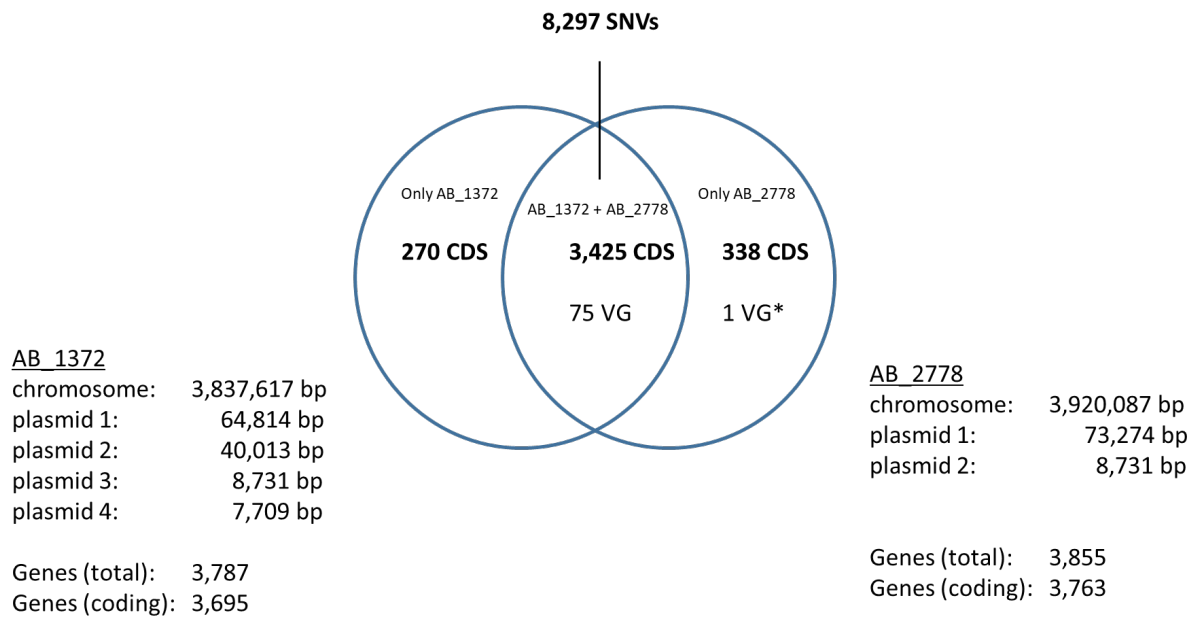
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40 **FIG S2** Presence-absence matrix of antibiotic resistance genes. Green areas indicate the  
 41 presence of the resistance gene in the respective genome. ST<sup>Pas</sup> = sequence type according  
 42 to Pasteur scheme.

Virulence gene	Isolate													
	AB_1494	AB_1523	AB_4514	AB_2169	AB_2778	AB_705	AB_1945	AB_4401	AB_1784	AB_1372	AB_3378	AB_2219	AB_2597	AB_3038
abu NC_010410.1:3570601-3571947														
tuf JFYLO1000029.1:1-790														
acyl carrier protein NC_009085.1:137227-137487														
hcp <i>Acinetobacter baumannii</i> strain M2														
tssM CP012004.1:2441902-2445726														
pilC CP010781.1:413401-414627														
pmrB CP017656.1:3261157-3262491														
pmrA CP045110.1:3277435-3278109														
toIC CP003856.1:554283-555629														
cspG AF022836.1:1286862-1287074														
tonB CP007712.1:3472653-3473408														
uspA CP032055.1:261889-262740														
bamE CP045110.1:933362-933760														
rpmF A1S_0816 VBIACiBau103176_0835														
gspM CP017656.1:2633021-2633500														
gspL CP017656.1:2633500-2634639														
gspC CP017656.1:313797-314633														
zur CP045110.1:237352-237849														
csuAB ATCC_17978:138967-1390403														
PgaABCD ATCC_17978-mff:1446525-1453251														
bmfS ATCC_17978-mff:3082462-3083889														
chpA ATCC_17978-mff:712598-717118														
antA AP022836.1:1879194-1879964														
hlyD AP022836.1:3181120-3182253														
adeAB ATCC_17978-mff:1931387-1935684														
pilQ CP003967.2:3349027-3351192														
pilY_2 CP008706.1:339222-343076														
csuA CP010781.1:2524132-2524680														
pilB CP010781.1:414657-416369														
gacS CP010781.1:683730-686537														
sodB CP012004.1:1256024-1256650														
plc1 CP012004.1:1575962-1578190														
bauA CP012004.1:158484-160673														
omp33 CP012004.1:211272-212171														
surA1 CP012004.1:2349708-2350025														
plc2 CP012004.1:3792688-3794856														
arpA CP012004.1:40488-41588														
arpB CP012004.1:41591-44716														
pid2 CP012004.1:522782-524407														
pid3 CP012004.1:627064-628527														
ompA CP012004.1:680965-682035														
secA CP014266.1:3309827-3312550														
lpxC CP017656.1:3818273-3819175														
gspD CP026761.1:1220009-1222279														
gspF CP026761.1:1332423-1333628														
gspE CP026761.1:1612778-1614268														
lpxA CP041587.1:2213618-2214406														
recA CP045110.1:2400486-2401535														
gacA CP045110.1:315918-316553														
pilA CP045110.1:603448-604248														
nduA CP045428.1:776957-777595														
lpxD CP045541.1:2929272-2930342														
basJ CP046654.1:201384-202553														
basI CP046654.1:202678-203433														
basH CP046654.1:203444-204178														
barB CP046654.1:204251-205846														
barA CP046654.1:205843-207426														
basG CP046654.1:207699-208850														
basF CP046654.1:208968-209837														
basD CP046654.1:211645-214587														
basC CP046654.1:214635-215945														
bauB CP046654.1:218396-21936														
bauE CP046654.1:219371-220141														
bauC CP046654.1:220138-221085														
bauD CP046654.1:221085-222068														
basB CP046654.1:222658-224685														
basA CP046654.1:224756-226603														
hlYB UFJZ01000001.1:1573874-1574581														
fbA-CII A1S_1544 VBIACiBau103176_1577														
atpA A1S_0153 VBIACiBau103176_0169														
purH A1S_2188 VBIACiBau103176_2241														
pilT fig 470.1295.peg.331 X87_01670														
catalase fig 470.1295.peg.4046 X87_20560														
argG ABSDF1378 VBIACiBau88365_1334														
sucC VBIACiPar182842_0269														
VBIACiUrs186641_3450														
zigA Lcl A1S_3411 B4151365														

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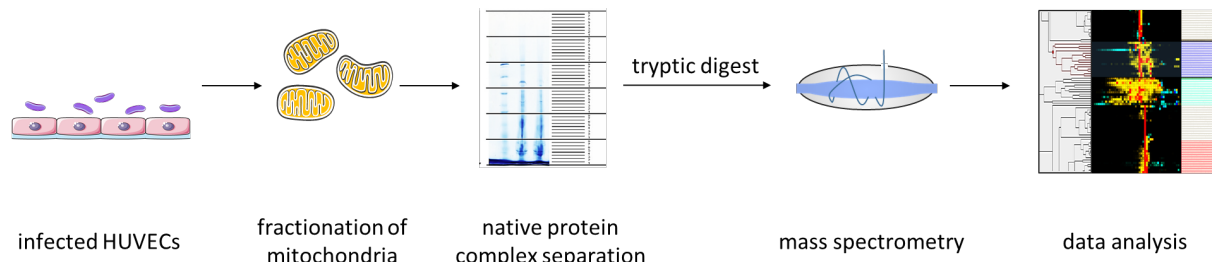
**FIG S3** Presence-absence matrix of virulence genes. Green areas indicate the presence of the resistance gene in the respective genome. ST<sup>Pas</sup> = sequence type according to Pasteur scheme.



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49 **FIG S4** Comparison of genomes between AB\_1372 and AB\_2778. SNVs, single nucleotide  
 50 variants; CDS, coding gene sequences; VG, virulence genes; VG\*, acyl carrier protein  
 51 NC\_009085.1:137227-137487.

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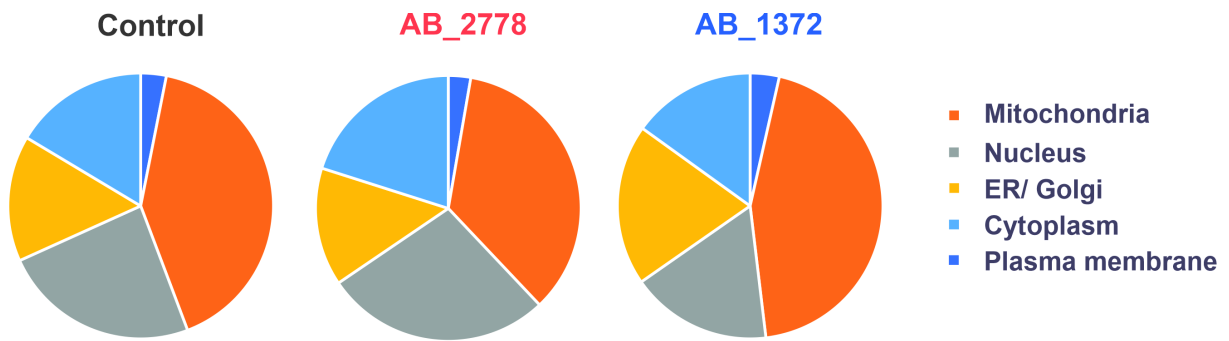
54 **FIG S5** The workflow of the complexome analysis is depicted. Mitochondria of *A. baumannii*

55 infected HUVECs were fractionated and proteins were separated by blue native page. The gel

56 was sliced into 48 pieces followed by a tryptic digest and mass spectrometry. Migration profiles

57 of identified proteins were analysed and protein complexes were clustered using NOVA.

58



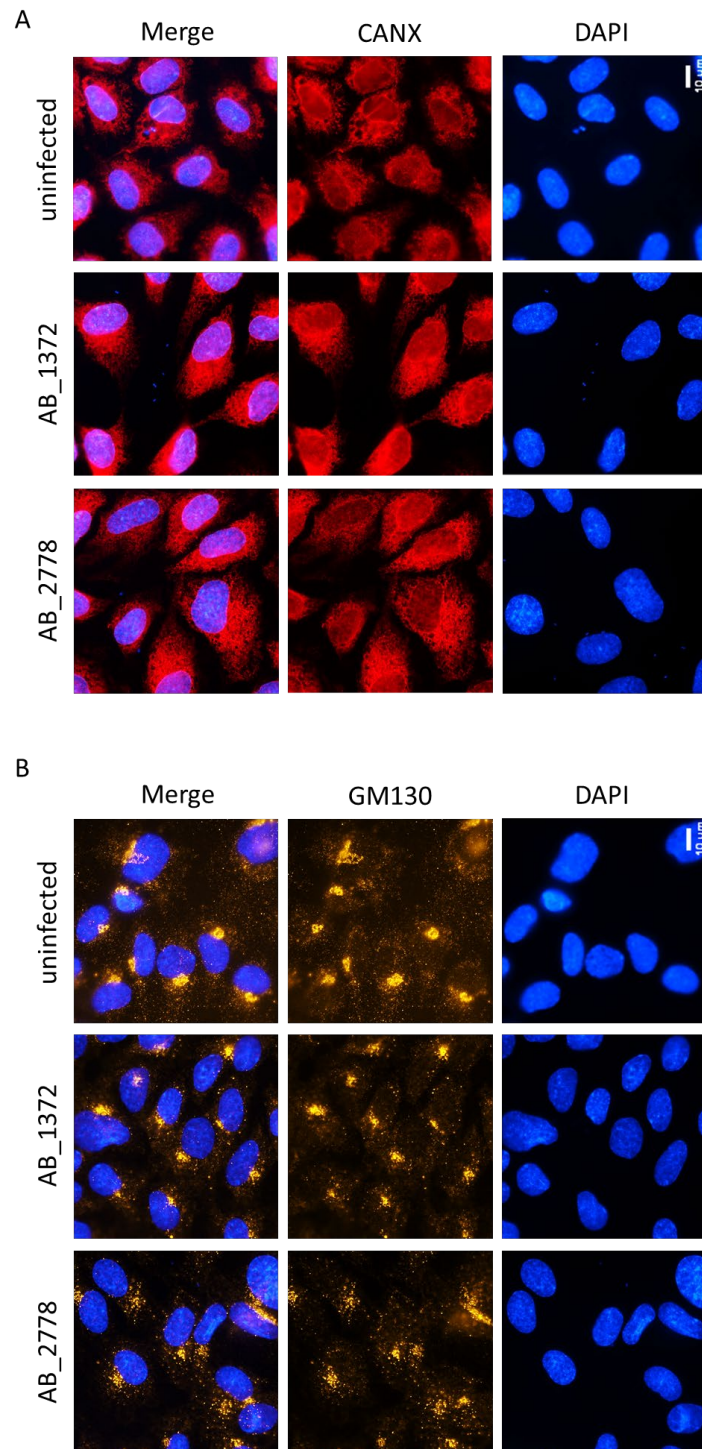
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61 **FIG S6** The gene ontology term was assigned to each protein from complexome profiling. The  
 62 proportions of each cell compartment were plotted for the uninfected control and for HUVECs  
 63 infected with AB\_2778 and AB\_1372, respectively.

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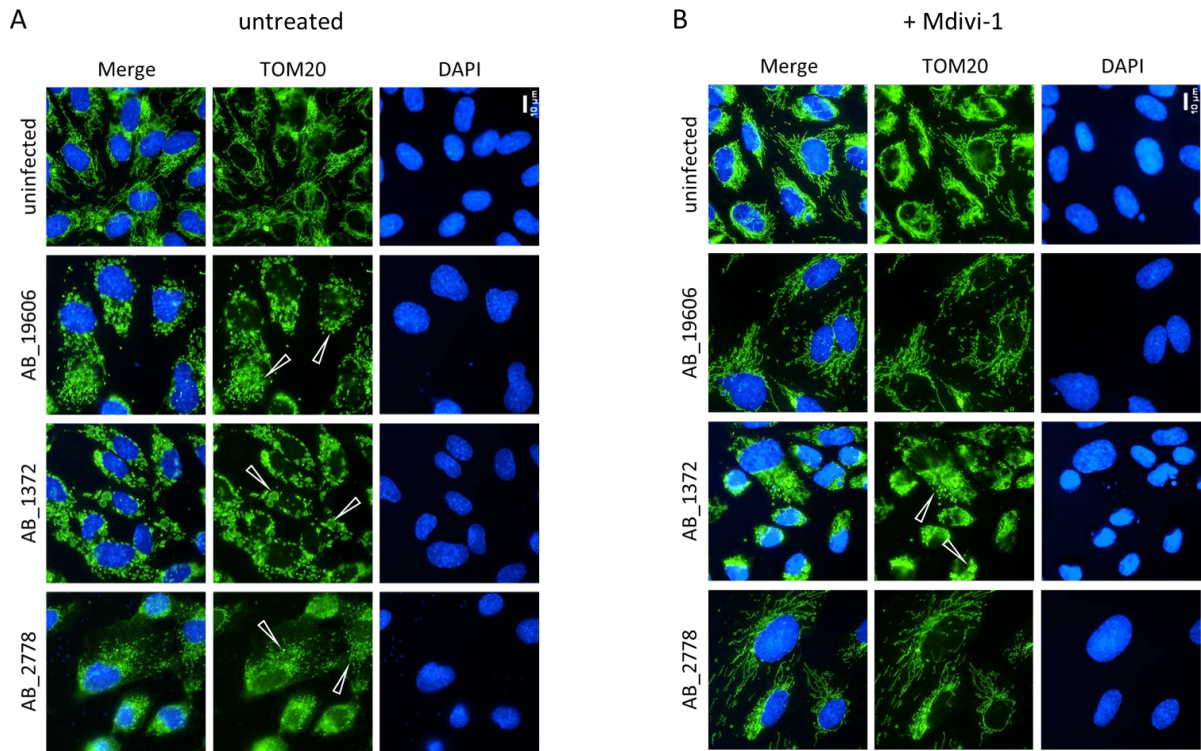


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68 **FIG S7** Analysis of morphology of endoplasmic reticulum (A) and Golgi apparatus (B) upon  
69 bacterial infection. HUVECs were infected with AB\_1372 or AB\_2778 for 16 hours (MOI 50).  
70 Representative immunofluorescence images of either uninfected and infected HUVECs. DAPI  
71 staining was used for visualisation of DNA (blue). The endoplasmic reticulum marker protein  
72 CANX (rot) and the Golgi apparatus marker protein GM130 (gold) were immunostained and  
73 morphology was monitored (scale bar: 10  $\mu$ m).

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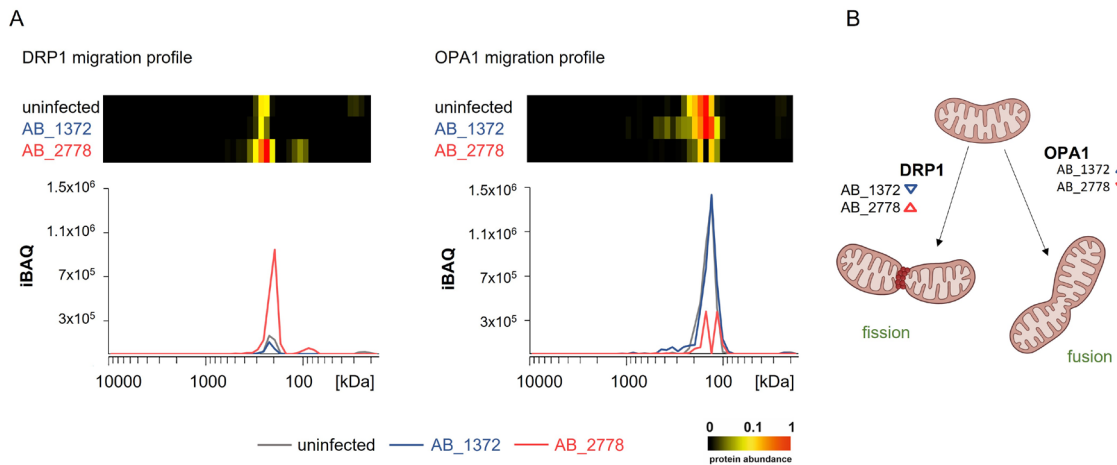




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77 **FIG S8** Impact of the inhibitor Mdivi-1 on mitochondrial morphology upon bacterial infection.  
 78 (A) Pictures depict changes in morphology of mitochondria upon infection with AB\_1372,  
 79 AB\_2778 and AB\_19606, respectively, 10 hpi (MOI 50). (B) Representative  
 80 immunofluorescence images of uninfected and infected HUVECs after pre-treatment with  
 81 25 μM Mdivi-1 (DRP-1 inhibitor) 14 hours prior to infection. DAPI staining was used for  
 82 visualisation of DNA (blue). The outer mitochondrial membrane protein TOM20 (green) was  
 83 immunostained and mitochondrial morphology and dynamics were monitored (scale bar:  
 84 10 μm). Triangles in TOM20 images indicate morphological changes of mitochondria  
 85 compared to uninfected control.

86

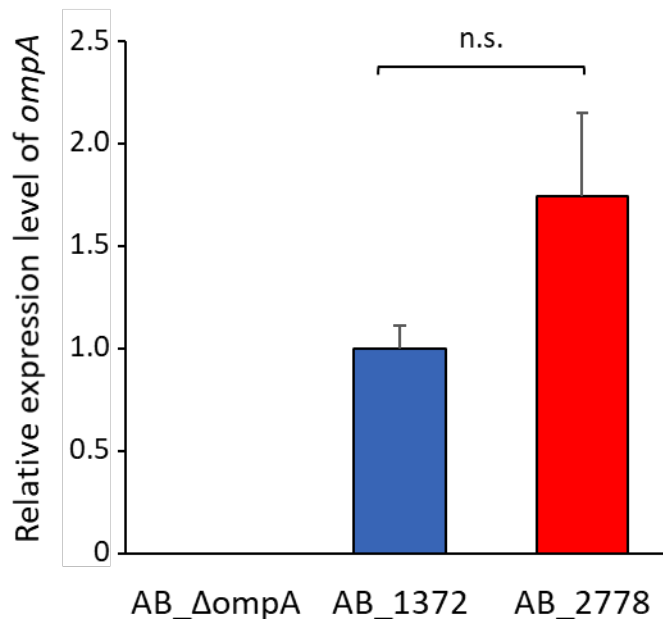


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89 **FIG S9** Abundance of DRP1 and OPA1 upon bacterial infection (A) Heat map of normalized  
 90 protein abundance distribution and migration profile of absolute protein abundance of DRP1  
 91 and OPA1 upon infection with AB\_1372 and AB\_2778, respectively and uninfected HUVECs.  
 92 (B) Direction of triangles indicate abundance of OPA1 and DRP1 in infected HUVECs for the  
 93 AB\_1372 and AB\_2778, respectively.

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98 **FIG S10** Relative expression levels of *ompA* in AB\_1372, AB\_2778 and AB\_ΔompA (internal  
99 control) isolated from HUVECs after infection. Total RNA was isolated and relative levels of  
100 gene expression were measured by qRT-PCR using the housekeeping gene *rpoB*. The relative  
101 expression of *ompA* was normalized to AB\_1372. Values are means ± SEM derived from two  
102 independent experiments with each four technical replicates. Statistical analysis was  
103 performed by a two-tailed t-test. n.s., not significant.

104 **Supplementary Table**

105

106 **TABLE S1** Ratio of *in vitro* cytotoxicity and *in vivo* virulence, calculated by dividing the  
 107 percentage of apoptotic HUVECs in Annexin V/PI assay and log(LD<sub>50</sub>) from *G. mellonella* time-  
 108 kill experiments.

Isolate	Apoptotic HUVECs[%]/log(LD <sub>50</sub> )	Apoptotic HUVECs[%]	Log(LD <sub>50</sub> )
AB_1372	0.76 ± 0.33	4.59 ± 1.22	6.05 ± 0.14
ATCC 19606 <sup>T</sup>	1.46 ± 0.63	7.38 ± 2.14	5.07 ± 0.18
AB_1523	2.45 ± 0.86	12.24 ± 3.24	4.99 ± 0.18
AB_3378	2.47 ± 0.20	12.78 ± 1.52	5.18 ± 0.34
AB_2597	2.68 ± 0.44	11.65 ± 2.27	4.35 ± 0.11
AB_1784	2.82 ± 0.24	10.86 ± 1.57	3.85 ± 0.20
AB_2219	2.94 ± 1.08	13.05 ± 3.73	4.43 ± 0.22
AB_4514	2.95 ± 0.26	12.03 ± 1.76	4.08 ± 0.15
AB_2169	2.98 ± 0.18	11.73 ± 1.42	3.94 ± 0.14
AB_5075	3.14 ± 0.09	17.37 ± 1.20	5.53 ± 0.19
AB_1494	3.27 ± 0.47	15.36 ± 2.67	4.70 ± 0.12
AB_4401	3.38 ± 0.03	14.47 ± 0.67	4.29 ± 0.13
AB_3038	3.81 ± 0.45	15.51 ± 2.62	4.07 ± 0.16
AB_705	3.87 ± 0.16	18.45 ± 1.65	4.77 ± 0.20
AB_1945	4.28 ± 0.32	18.02 ± 2.37	4.21 ± 0.20
AB_2778	5.50 ± 0.44	22.58 ± 3.12	4.11 ± 0.12

109

## 110 **Supplementary methods**

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### 112 **Genome sequencing**

113 Library preparation for Nanopore sequencing was done using the SQK-RBK004 rapid  
114 barcoding kit. Sequencing was performed on a MinION MK1B sequencer utilizing R9.4.1 flow  
115 cells. Raw signal data was base called and demultiplexed using the high accuracy base calling  
116 model of guppy basecaller version 4.0.11. Raw data was filtered using NanoFilt for long reads  
117 resulting in datasets of reads with an average genome coverage of at least 30-fold for long  
118 reads. *De novo* hybrid assembly was conducted using Unicycler version 0.4.8 utilizing the bold  
119 assembly mode. Gene presence-absence plots were drawn utilizing a python script  
120 (accessible via [https://raw.githubusercontent.com/sanger-](https://raw.githubusercontent.com/sanger-pathogens/Roary/master/contrib/roary_plots/roary_plots.py)  
121 [pathogens/Roary/master/contrib/roary\\_plots/roary\\_plots.py](https://raw.githubusercontent.com/sanger-pathogens/Roary/master/contrib/roary_plots/roary_plots.py)).

122

### 123 **Growth kinetics of *A. baumannii***

124 To compare growth of AB\_1372 and AB\_2778, bacteria cultures were adjusted to an optical  
125 density (OD<sub>600</sub>) of 0.05 in lysogeny broth (LB). A total of 200 µL of the bacterial solutions were  
126 transferred to 96-well plates and incubated in a plate reader (infinite M200 Pro, TECAN) for  
127 24 h at 37 °C. OD<sub>600</sub> was measured every 20 min with a shaking amplitude of 3 mm during  
128 measurement.

129

### 130 **Biofilm analysis**

131 Bacteria were cultivated in tryptic soy broth (TSB) medium without dextrose at 37 °C overnight.  
132 Subsequently, a culture with TSB medium with 2% (w/v) dextrose was inoculated and  
133 cultivated until an OD<sub>600</sub> of 0.5 was reached. A 1:20 dilution of the culture (or medium as  
134 negative control) was transferred into a 96-well microtitre plate (Nunclon Delta Surface,  
135 Thermo Fisher) and incubated for 24 h at 37 °C. The supernatant was discarded and the biofilm  
136 was washed three times with PBS. Subsequently, the plate was dried at 65 °C for 5 min. The  
137 biofilm was stained for two minutes using a saturated solution of crystal violet and washed five  
138 times with *A. dest.* Subsequently, ethanol (200 µl) was added to each well and absorption at  
139 405 nm (crystal violet) and 570 nm (reference) was measured with a plate reader (infinite M200  
140 Pro, TECAN).

141

### 142 **Analysis of bacterial adhesion to human endothelial cells**

143 HUVECs were seeded into 6-well plates and infected with *A. baumannii* (MOI 200). After  
144 infection, the 6-well plates were incubated for 1 h. The supernatant was removed and cells  
145 with adherent bacteria were washed with PBS and detached using a cell scraper. Thereafter,  
146 adherent bacteria were quantified by plating serial dilution series. Visualisation of bacterial

147 adhesion to human endothelial cells was done by fluorescence microscopy. For this purpose,  
148 HUVECs were seeded into 6-well plates containing collagenized coverslips and infected with  
149 *A. baumannii* (MOI 200) for 2 h. Before infection, bacteria were stained with carboxyfluorescein  
150 succinimidyl ester (CFSE) (diluted 1:300 in 1 mL *A. dest.*) for 30 min (37 °C). After incubation,  
151 cells were washed to remove non-adherent bacteria and fixed with 400 µL 3.75%  
152 paraformaldehyde (4 °C) for 15 min. Cells were thereafter incubated with 0.2% Triton X-100  
153 for 15 min at room temperature. The incubation was followed by staining the cells with 500 µL  
154 tetramethylrhodamine (TRITC)-phalloidin (1:500) for 1 h at room temperature and 4',6-  
155 diamidino-2-phenylindole (DAPI) for 10 min at 4 °C. Bacterial adhesion to HUVECs was then  
156 visualised by fluorescence microscopy (Nikon Eclipse Ci-L).

157

### 158 **Analysis of caspase activity**

159 Activity of caspase-3, caspase-7, caspase-8 and caspase-9 were determined in 96-well plates  
160 infected with *A. baumannii* (MOI 10) using the Caspase-Glo® 3/7 Assay and the Caspase-Glo®  
161 8 and 9 Assay (Promega, Walldorf, Germany). After infection, the 96-well plates were  
162 incubated for 2, 4, 6, 8, 16 and 24 h (37 °C, 5% CO<sub>2</sub>). After incubation, 10 µL of the caspase-  
163 3/7 or caspase-8 or caspase-9 solution were added to each well. The plates were mixed for  
164 30 s (300 rpm) in a plate reader (infinite M200 Pro, TECAN) and incubated for 45 min.  
165 Thereafter, plates were mixed again for 30 s (300 rpm) and luminescence was recorded.

166

### 167 **Evaluation of bacterial virulence using the *Galleria mellonella* in vivo infection model**

168 Larvae of the greater wax moth (*Galleria mellonella*) with a weight of 150-250 mg were  
169 obtained from UK waxworms (Sheffield, UK). *A. baumannii* isolates were grown overnight in  
170 LB and then diluted 100-fold into fresh LB and grown for 3 h. After two washes with phosphate-  
171 buffered saline (PBS), bacteria were resuspended in PBS to a final OD<sub>600</sub> of 1.0 and 10-fold  
172 serial dilutions were prepared in PBS. Each experiment included control groups of non-injected  
173 larvae or larvae injected with PBS. Larvae were considered dead if they did not respond to  
174 physical stimuli. Generation of time-kill curves and calculation of the median lethal dose (LD<sub>50</sub>)  
175 after 24 h was done by non-linear regression analysis using GraphPad Prism as described  
176 (49, 50, 54). Experiments were repeated three times using ten larvae per experimental group.

177

### 178 **Determination of *ompA* expression levels**

179 Quantification of *ompA* gene expression was done as described previously (58). Total RNA  
180 was isolated from bacteria harvested after infection of HUVECs (Monarch Total RNA Miniprep  
181 Kit, NEB, Frankfurt am Main, Germany). Subsequently, samples were used for quantitative  
182 real-time PCR (qRT-PCR). Expression levels of *rpoB* as internal control (*rpoB*\_RT\_fwd primer:  
183 GAG TCT AAT GGC GGT GGT TC; *rpoB*\_RT\_rev Primer: ATT GCT TCA TCT GCT GGT TG)

184 and *ompA* (*ompA*\_RT\_F primer: AGG TCA CAC AGA TAA CAC TGG; *ompA*\_RT\_R primer:  
185 AAC GTT GTA TTC GTT TAC AAG AGC) (Luna, Universal One-Step RT-qPCR Kit, NEB,  
186 Frankfurt am Main, Germany) were quantified (11). The relative expression of *ompA* in bacteria  
187 after infection was normalized to planktonic bacteria.

188