

1 **SUPPLEMENTAL MATERIAL**

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3 **Table S1.** Summary of proteins detected in the supernatant fraction of *B. thailandensis* wildtype–vir  
4 and *tssK-5*–vir cultures by mass spectrometry.

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6 **Fig S1.** The *B. thailandensis clpV-5*–GFP mutant induces host cell fusions. Representative  
7 fluorescence microscopy images of RAW 264.7 macrophages infected with *B. thailandensis clpV-5*–  
8 GFP and wildtype and  $\Delta T6SS-5$  constitutively expressing GFP. The host cell plasma membrane was  
9 stained with Alexa Fluor 594–WGA conjugate and DNA was visualized with DAPI. Scale bar, 10  
10  $\mu\text{m}$ .

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12 **Movie S1.** Representative time lapse fluorescence microscopy images of ClpV–5–GFP foci within  
13 *B. thailandensis* during infection of HeLa cells at 17 h post-infection (10 s intervals shown at seven  
14 frames per second).

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16 **Movie S2.** Representative time lapse fluorescence microscopy images of ClpV–1–GFP foci within  
17 *B. thailandensis* grown in LB and transferred to agarose pads (10 s intervals shown at seven frames  
18 per second).

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26 Table S1.

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Lous tag	Annotation <sup>a</sup>	Average spectral counts <sup>b</sup>			Average no. of unique peptides <sup>c</sup>		
		wt <sup>d</sup>	wt-vir	$\Delta$ tssK-5-vir	wt <sup>d</sup>	wt-vir	$\Delta$ tssK-5-vir
<b>BTH_I0868</b>	<b>Hcp protein</b>	<b>N/D</b>	<b>326</b>	<b>43.5</b>	<b>–</b>	<b>12</b>	<b>7</b>
BTH_I0112	Lipoprotein, putative	158.7	181	178.5	14	13	13.5
BTH_I1925	Chitin binding domain protein	403.2	120.5	105	21.2	14	12.5
BTH_I2945	Peptidase, M1 family	139	119	127	22.7	21	20.5
BTH_I3070	Translation elongation factor Tu	109.2	105.5	119.5	17	13.5	16.5
BTH_I1578	Microbial collagenase, putative	199.7	86.5	92.5	24	13.5	13.5
BTH_I2962	Unnamed protein product: Similar to Hcp protein	418	80.5	78	12.7	8	7.5
BTH_I3071	Translation elongation factor 2 (EF-2/EF-G)	65.7	73.5	64.5	18.7	19	17.5
BTH_I1421	Serine-type carboxypeptidase family protein	84.2	68.5	63.5	15	12	12.5
BTH_I3196	Flagellin	29.2	66.5	64.5	6	6	5.5
BTH_I0816	ZmpA-like peptidase, Metallo peptidase	N/C	61	68.5	0.7	13	15
<b>BTH_I0854</b>	<b>Ubiquitin-specific proteinase 31, putative</b>	<b>N/D</b>	<b>60.5</b>	<b>66.5</b>	<b>–</b>	<b>20</b>	<b>21</b>
BTH_I1544	Flagellar hook-associated protein 2	44.7	58.5	56	8	13.5	13.5
BTH_I3300	Amino acid/amide ABC transporter substrate-binding protein, HAAT family	20	57	66.5	7	10.5	11
BTH_I0113	PqA	44	53	53.5	17	13	12
BTH_I1225	L-glutamate-binding protein / L-aspartate-binding protein	N/C	52.5	34.5	6.2	13	9
BTH_I2402	Chitinase family 18	333.2	49.5	47	9	5	6
BTH_I0658	Malate dehydrogenase	33	48.5	53	10	12.5	12.5
BTH_I0783	Hypothetical protein	186.2	47	49.5	12.7	6.5	7.5
BTH_I1633	Hypothetical protein	28.2	46.5	40.5	7.7	9.5	7.5
BTH_I0646	Succinyl-CoA synthase, beta subunit	30	46	49	8.2	12.5	12
BTH_I1306	Carbohydrate ABC transporter substrate-binding protein, PAAT1 family	80	45.5	63.5	14.2	11	13
BTH_I0854	Aconitase	N/C	43.5	48	6.2	12	13.5
BTH_I1458	Chaperonin GroEL	112	43	49.5	21.2	10	10.5
BTH_I1834	LasA protease precursor	205	39.5	58	15	7.5	9.5
<b>BTH_I0863</b>	<b>Rhs element Vgr protein</b>	<b>N/D</b>	<b>38</b>	<b>N/D</b>	<b>–</b>	<b>13.5</b>	<b>–</b>
BTH_I1598	Amino acid/amide ABC transporter substrate-binding protein, HAAT family	N/C	34.5	24.5	0.7	8.5	6.5
BTH_I0517	Hypothetical protein	66.7	34	33.5	10.2	8	6.5
BTH_I1069	gp28	46	33	34	14	5.5	6
BTH_I1282	Catalase/peroxidase HPI	N/C	32.5	41	1.2	11	10.5
BTH_I1783	Amino acid ABC transporter substrate-binding protein, PAAT family	22.7	31.5	34.5	6.7	8.5	8.5
BTH_I1872	Levansucrase	60.2	31	35.5	14.7	9	8
BTH_I2276	Putrescine ABC transporter, periplasmic putrescine-binding protein	N/C	30.5	26.5	3.2	10	8
BTH_I0955	Heat shock protein HtpG	33.2	30.5	32.5	11.5	10.5	9
BTH_I0233	Lytic murein transglycosylase, putative	28.7	30	32	10.2	10	9.5
<b>BTH_I3225/I0089<sup>f</sup></b>	<b>Rhs element Vgr protein, putative</b>	<b>62.7</b>	<b>28.5</b>	<b>29</b>	<b>19.2</b>	<b>9</b>	<b>10</b>
BTH_I1638	SSU ribosomal protein S1P	26.67	28	24	10.7	5.5	6
BTH_I0759	Isoctrate dehydrogenase (NADP)	18.2	27.5	27	6	6.5	6
BTH_I02037	Aromatic amino acid aminotransferase apoenzyme (EC 2.6.1.57)	16.2	26.5	27	6.7	9	8.5
<b>BTH_I3226/I0090<sup>e</sup></b>	<b>Hypothetical protein</b>	<b>57.33</b>	<b>26.5</b>	<b>22</b>	<b>11</b>	<b>10</b>	<b>4</b>
BTH_I1654	Electron transfer flavoprotein, alpha subunit	16.2	26	27.5	5	5.5	6
BTH_I1457	Chaperonin, 10 kDa	20.7	26	30.5	5.7	5.5	4.5
BTH_I0675	Serine protease	N/C	24.5	25	0.7	6.5	7
BTH_I0663	Phosphoglycerate kinase	N/C	24	25	2.7	4.5	5.5
BTH_I2028	Translation elongation factor Ts (EF-Ts)	18.2	24	20.5	6	6.5	5.5
BTH_I1851	Metallopeptidase domain protein	61	24	26	11.2	6	6
BTH_I0564	Acyl-CoA dehydrogenase domain protein	15	23.5	18.5	5.7	7.5	6
BTH_I1852	Leucyl aminopeptidase (EC 3.4.11.10), Metallo peptidase.	37.2	22.5	23	10.5	6	7
BTH_I0380	Serine peptidase	88.2	20.5	28	18.7	6	10
BTH_I0647	Succinyl-CoA synthetase (ADP-forming) alpha subunit	15	19.5	24.5	4.7	5.5	5.5
BTH_I0967	Cytochrome c family protein	23	19.5	19	5	3.5	3.5
BTH_I1515	Hypothetical protein	157	19.5	23.5	24.2	7	8
BTH_I1308	Chaperone protein DnaK	19	18	16	7	6	4.5
BTH_I2231	Nucleoside diphosphate kinase	18	16.5	21.5	4	3	3.5
BTH_I2092	Antioxidant, AhpC/Tsa family	27.7	15.5	18	6.2	5	5
BTH_I0720	Hypothetical protein	25	N/C	N/C	5.7	2.5	4
<b>BTH_I0068</b>	<b>Hypothetical protein</b>	<b>32</b>	<b>N/C</b>	<b>N/C</b>	<b>6.7</b>	<b>2</b>	<b>3</b>
BTH_I1243	Putative ABC transporter ATP-binding protein	43.7	N/C	N/C	11	2.5	2
<b>BTH_I2697</b>	<b>Rhs element Vgr protein, putative</b>	<b>45.2</b>	<b>N/C</b>	<b>N/C</b>	<b>13</b>	<b>5</b>	<b>4.5</b>
BTH_I2044	Serine protease, subtilase family	53	N/C	N/C	14	1.2	1
BTH_I1933	Hypothetical protein	86.7	N/C	N/C	3.2	0.7	0.7
<b>BTH_I2723</b>	<b>Filamentous haemagglutinin</b>	<b>25.32</b>	<b>N/D</b>	<b>N/D</b>	<b>9.2</b>	<b>–</b>	<b>–</b>
<b>BTH_I2691</b>	<b>Hypothetical protein</b>	<b>27</b>	<b>N/D</b>	<b>N/D</b>	<b>9</b>	<b>–</b>	<b>–</b>
BTH_I0261	Hypothetical protein	30	N/D	N/D	7.7	–	–
<b>BTH_I2705</b>	<b>Rhs element Vgr protein</b>	<b>38</b>	<b>N/D</b>	<b>N/D</b>	<b>13</b>	<b>–</b>	<b>–</b>
<b>BTH_I2693</b>	<b>Rhs element Vgr protein, putative</b>	<b>39</b>	<b>N/D</b>	<b>N/C</b>	<b>12</b>	<b>–</b>	<b>0.7</b>

<sup>a</sup> Burkholderia Genome Database (Burkholderia.com)<sup>b</sup> Average number of spectral counts detected across all technical replicates<sup>c</sup> Average number of unique peptide sequences obtained from biological replicates<sup>d</sup> Reference wild type secretome (Russell et al. 2012)<sup>e</sup> BTH\_I3226 and BTH\_I0090 encode proteins with 96% sequence identity. Unique peptides from the variable regions of the proteins were detected; however, using mass spectrometry it is not possible to define their relative contributions.<sup>f</sup> BTH\_I3225 and BTH\_I0089 encode 100% identical VgrG proteins. By mass spectrometry it is not possible to discriminate between the two proteins.

N/C, not considered.

N/D, not detected

T6SS-1 secreted proteins

T6SS-5 secreted proteins

Secretion is induced by VirAG but occurs independently of T6SS-5.

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52 Figure S1.

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