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**Antimicrobial peptide cWFW kills by combining lipid phase separation with autolysis**

Supplementary Information

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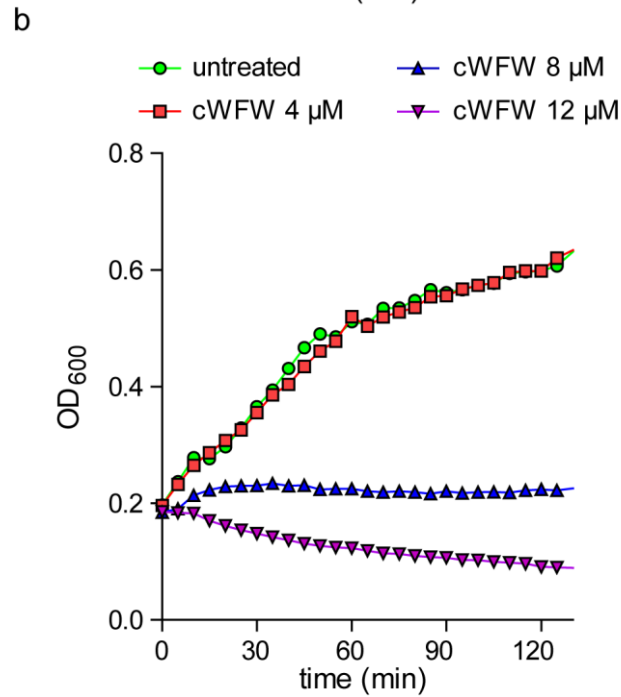
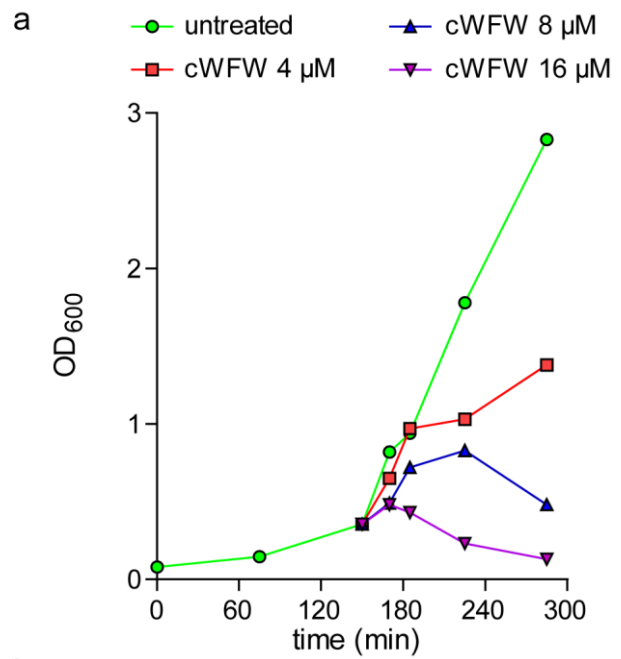
Content:

Supplementary Figures 1-3

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Supplementary Movie 1

Supplementary References



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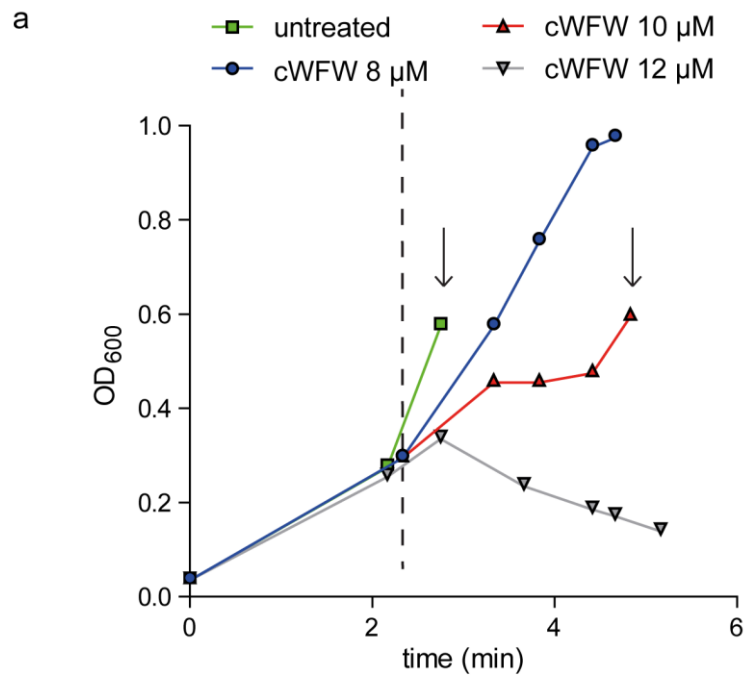
25 **Supplementary Fig. 1: Concentration-dependent growth inhibition of *B. subtilis* in the**  
 26 **different growth media used in this study**

27 (a) Growth curves of *B. subtilis* 168/DSM402 at 37 °C in BMM upon addition of different  
 28 concentrations of cWFW. The time point of cWFW addition is indicated with a dashed line.

29 The selected inhibitory concentration (8 μM) was used for proteome and ion content analysis.

30 (b) Growth curves of *B. subtilis* 168 at 37 °C in LB-medium in the presence of different

31 concentrations of cWFW as measured on microtiter plates. The growth inhibition upon peptide  
32 addition to a logarithmically growing culture at  $OD_{600}=0.2$  is shown. The graphs depict a  
33 representative of three independent measurements.



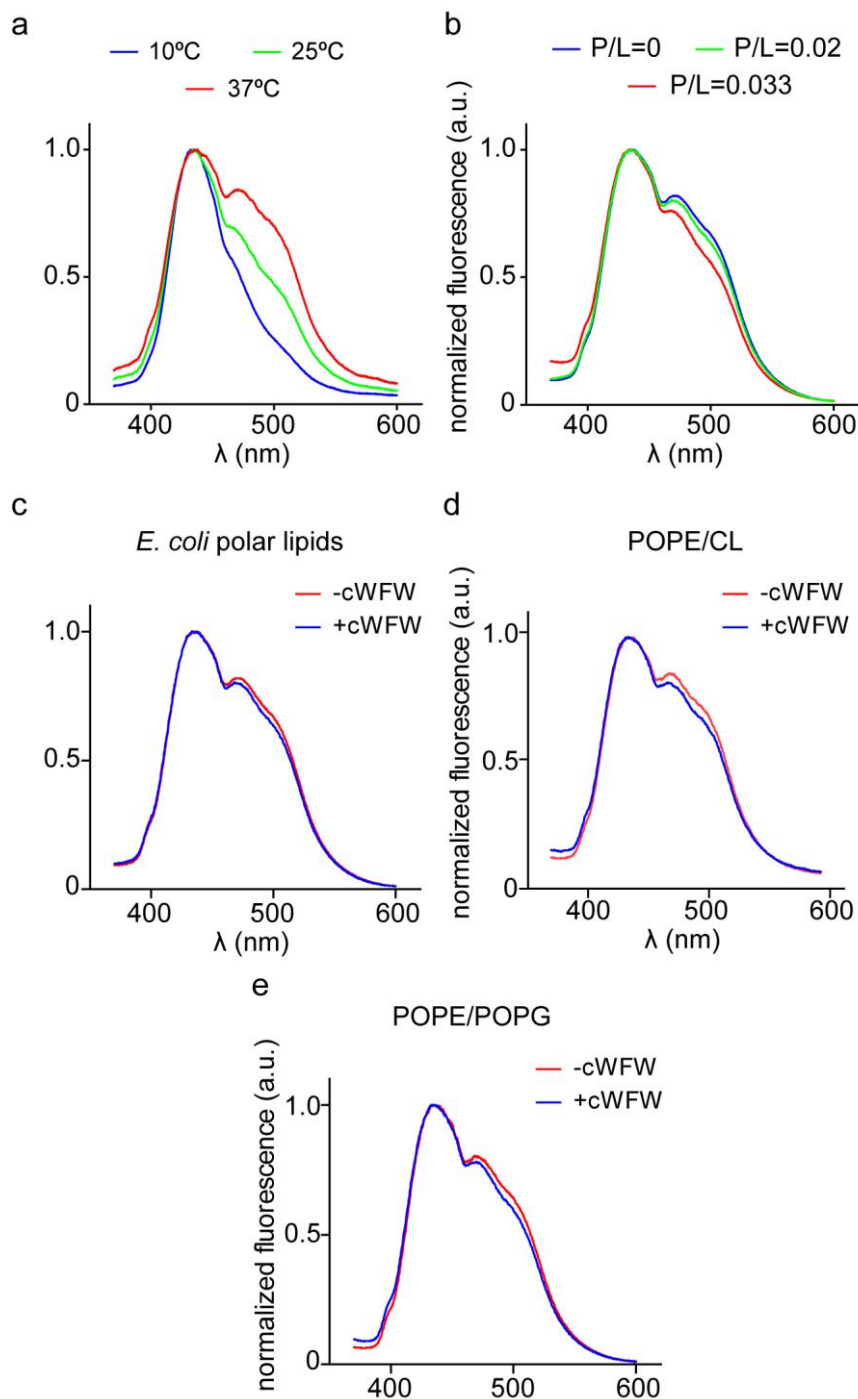
b

|                           | untreated   | 10 μM cWFW (2.5h) |
|---------------------------|-------------|-------------------|
| C <sub>14:0</sub> iso     | 1.5         | 2.0               |
| C <sub>14:0</sub>         | 0.5         | 0.55              |
| C <sub>15:0</sub> iso     | 18.0        | 23.8              |
| C <sub>15:0</sub> anteiso | 37.1        | 37.3              |
| C <sub>16:0</sub> iso     | 5.4         | 5.8               |
| C <sub>16:0</sub>         | 4.9         | 4.7               |
| C <sub>17:0</sub> iso     | 15.0        | 13.1              |
| C <sub>17:0</sub> anteiso | 16.0        | 11.3              |
| C <sub>18:0</sub>         | 1.2         | 0.8               |
| sum                       | 99.4        | 99.4              |
| saturated                 | 6.5         | 6.1               |
| iso                       | 39.8        | 44.8              |
| anteiso                   | 53.1        | 48.6              |
| iso/anteiso               | <b>0.75</b> | <b>0.92</b>       |
| short (<C <sub>16</sub> ) | 57.0        | 63.6              |
| long (≥C <sub>16</sub> )  | 37.0        | 30.0              |
| short/long                | <b>1.54</b> | <b>2.12</b>       |

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35 **Supplementary Fig. 2: Membrane fatty acid adaptation in response to cWFW**

36 (a) Growth curves of *B. subtilis* 168 at 37 °C in LB medium upon addition of different  
 37 concentrations of cWFW. The time points of cell harvest for fatty acid analysis are indicated  
 38 with arrows. The graphs depict a representative of two independent measurements. (b) The  
 39 cellular fatty acid profile in the absence of cWFW and after 2.5 h incubation with 10 μM  
 40 cWFW. Strain used: *B. subtilis* 168 (wild type).



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42 **Supplementary Fig. 3: cWFW-dependent laurdan fluorescence emission spectra in LUVs**  
 43 **of varying lipid composition**

44 (a) Fluorescence emission spectrum of laurdan incorporated into large unilamellar vesicles  
 45 (LUVs) formed of *E. coli* polar lipid extract at different temperatures. Note the spectral shift  
 46 caused by reduced membrane fluidity at lower temperatures. (b) Fluorescence emission  
 47 spectrum of laurdan stained LUVs formed of *E. coli* polar lipids at 37 °C in the presence of

48 different concentrations of cWFW. P/L indicates peptide-to-lipid molar ratio. (C-D)  
49 Fluorescence emission spectrum of laurdan stained LUVs formed of *E. coli* polar lipids, a  
50 binary mixture of POPE and cardiolipin (POPE/CL) and a binary mixture of POPE and POPG,  
51 respectively, in the absence and presence of cWFW (P/L=0.02). The spectra depict a  
52 representative of two independent measurements.

**Supplementary Table 1: Comparison of cWFW with proteome response library**

| protein                 | cWFW      | MP196 | vancomycin | mersacidin | gallidermin | nisin | gramicidin S | valinomycin | gramicidin A | ionomycin | triton X-100 |
|-------------------------|-----------|-------|------------|------------|-------------|-------|--------------|-------------|--------------|-----------|--------------|
| <b>YceC<sup>+</sup></b> | x         | x     | x          | x          | x           |       | x            |             |              | x         | x            |
| FosB                    | x         | x     |            |            |             |       |              |             |              |           |              |
| YfhM                    | x         | x     |            |            |             |       |              |             |              | x         | x            |
| YthP                    | x         | x     |            |            |             |       |              |             |              |           |              |
| YvlB                    | x         | x     |            |            | x           |       | x            | x           |              | x         | x            |
| <b>LiaH<sup>#</sup></b> | x         | x     |            | x          | x           | x     | x            |             |              |           |              |
| RacX                    | x         | x     |            |            |             |       | x            | x           |              |           |              |
| <b>YpuA<sup>#</sup></b> | x         |       |            | x          | x           | x     | x            |             |              |           |              |
| DltA                    | x         | x     |            |            | x           |       |              |             |              |           |              |
| <b>PspA<sup>°</sup></b> | x         | x     |            |            | x           | x     | x            | x           | x            |           | x            |
| YjdA                    | x         | x     |            |            |             |       | x            | x           |              |           |              |
| YoxD                    | x         | x     | x          |            |             |       | x            |             | x            |           |              |
| <b>NadE<sup>°</sup></b> | x         | x     |            |            | x           | x     | x            | x           | x            |           | x            |
| NfrA                    | x         | x     |            |            | x           |       | x            | x           |              |           |              |
| YwrO                    | x         | x     |            |            |             |       | x            |             |              |           | x            |
| <b>CitZ</b>             | x         | x     |            |            |             |       |              | x           |              | x         |              |
| GsiB                    | x         | x     |            |            |             |       | x            | x           |              |           |              |
| YdaG                    | x         | x     |            |            |             |       |              |             |              |           |              |
| Dps                     | x         | x     |            |            |             |       |              | x           |              |           |              |
| ClpP                    | x         |       |            |            |             |       | x            |             |              |           |              |
| Tpx                     | x         |       |            |            |             |       |              |             |              |           | x            |
| YqiW                    | x         |       |            |            |             |       |              |             |              | x         |              |
| YdbD                    | x         | x     |            |            |             |       | x            |             |              |           |              |
| SpoVG                   | x         | x     |            |            |             |       |              | x           |              |           | x            |
| <b>total markers</b>    | <b>52</b> | 36    | 5          | 13         | 25          | 8     | 21           | 22          | 5            | 38        | 26           |

54 \*specific marker for cell envelope stress, °specific marker for membrane stress, #specific marker for  
55 inhibition of membrane-bound cell wall biosynthesis steps <sup>1</sup>.

56 **Supplementary Table 2: Details of mass spectrometric protein spot identification (nLC-**  
 57 **ESI-MS)**

| <b>protein</b> | <b>mass weight [Da]</b> | <b>pI</b> | <b>PGLS score</b> | <b>number of matching proteins</b> | <b>coverage [%]</b> | <b>number of peptides with fragment data</b> | <b>precursor mass error [ppm]</b> | <b>product mass error [ppm]</b> |
|----------------|-------------------------|-----------|-------------------|------------------------------------|---------------------|--|-----------------------------------|---------------------------------|
| <b>AcsA</b>    | 64850                   | 5.54      | 4431              | 48                                 | 36                  | 48   | 1.38                              | 4.08                            |
| <b>ClpC</b>    | 90063                   | 5.75      | 22605             | 182                                | 60                  | 159  | 1.55                              | 4.88                            |
| <b>FosB</b>    | 17161                   | 6.14      | 754               | 4                                  | 17                  | 4  | 1.57                              | 2.17                            |
| <b>NfrA</b>    | 28302                   | 5.73      | 9602              | 24                                 | 33                  | 20   | 1.58                              | 4.45                            |
| <b>SalA</b>    | 38614                   | 5.23      | 6829              | 18                                 | 44                  | 33   | 1.42                              | 3.67                            |
| <b>YfhM</b>    | 32737                   | 6.07      | 7499              | 22                                 | 46                  | 20   | 1.23                              | 3.88                            |
| <b>YpuA</b>    | 31275                   | 4.51      | 20679             | 38                                 | 53                  | 30   | 1.75                              | 4.39                            |
| <b>GroEL</b>   | 57388                   | 4.53      | 35524             | 178                                | 90                  | 178  | 2.80                              | 7.77                            |
| <b>OhrB</b>    | 14591                   | 4.73      | 7866              | 23                                 | 94                  | 23   | 2.07                              | 9.24                            |
| <b>GtaB</b>    | 33049                   | 4.91      | 36499             | 111                                | 96                  | 111  | 2.18                              | 7.66                            |
| <b>TpiA</b>    | 27012                   | 4.79      | 1709              | 10                                 | 32                  | 10   | 2.21                              | 10.05                           |
| <b>PepF</b>    | 77024                   | 5.49      | 15612             | 116                                | 63                  | 116  | 1.63                              | 7.80                            |
| <b>PurH</b>    | 55719                   | 5.13      | 4098              | 60                                 | 51                  | 60   | 3.24                              | 8.39                            |
| <b>Ywjl</b>    | 33929                   | 5.06      | 15972             | 43                                 | 74                  | 43   | 2.01                              | 7.46                            |
| <b>FbaA</b>    | 30381                   | 5.02      | 12444             | 63                                 | 85                  | 63   | 2.06                              | 8.03                            |
| <b>MinD</b>    | 29388                   | 4.98      | 16778             | 43                                 | 62                  | 43   | 1.68                              | 7.57                            |
| <b>YkaA</b>    | 23829                   | 4.90      | 7090              | 27                                 | 63                  | 27   | 1.86                              | 9.71                            |

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59 **Supplementary Table 3: Details of mass spectrometric protein spot identification**  
60 **(MALDI-ToF/ToF)**

| <b>protein</b> | <b>mass weight [Da]</b> | <b>pI</b> | <b>mascot protein score</b> | <b>mascot protein score % C.I.</b> | <b>number of peptides used for identification</b> |
|----------------|-------------------------|-----------|-----------------------------|------------------------------------|---|
| <b>CitZ</b>    | 41702                   | 5.55      | 444                         | 100                                | 16  |
| <b>ClpP</b>    | 21668                   | 5.19      | 353                         | 100                                | 12  |
| <b>DltA</b>    | 55773                   | 5.10      | 276                         | 100                                | 13  |
| <b>Dps</b>     | 16583                   | 4.64      | 168                         | 100                                | 8   |
| <b>GlpK</b>    | 55045                   | 5.16      | 96                          | 100                                | 11  |
| <b>GroEL</b>   | 57388                   | 4.53      | 146                         | 100                                | 11  |
| <b>GsiB</b>    | 13789                   | 5.31      | 122                         | 100                                | 5   |
| <b>IspH</b>    | 34902                   | 5.68      | 91                          | 100                                | 2   |
| <b>LiaH</b>    | 25682                   | 6.20      | 112                         | 100                                | 13  |
| <b>LuxS</b>    | 17703                   | 5.29      | 301                         | 100                                | 5   |
| <b>NadE</b>    | 30376                   | 5.07      | 459                         | 100                                | 16  |
| <b>PspA</b>    | 25125                   | 5.87      | 91                          | 100                                | 11  |
| <b>RacX</b>    | 25270                   | 5.46      | 73                          | 100                                | 3   |
| <b>SodA</b>    | 22561                   | 5.33      | 414                         | 100                                | 8   |
| <b>SpoVG</b>   | 10886                   | 5.25      | 348                         | 100                                | 10  |
| <b>Tpx</b>     | 18204                   | 4.89      | 403                         | 100                                | 15  |
| <b>YceC</b>    | 21810                   | 5.46      | 224                         | 100                                | 14  |
| <b>YdaG</b>    | 15867                   | 5.33      | 111                         | 100                                | 5   |
| <b>YdbD</b>    | 30238                   | 5.06      | 103                         | 100                                | 10  |
| <b>YjdA</b>    | 27432                   | 5.74      | 55                          | 99                                 | 5   |
| <b>YoxD</b>    | 25283                   | 5.48      | 434                         | 100                                | 20  |
| <b>YqiW</b>    | 16186                   | 5.00      | 137                         | 100                                | 7   |
| <b>YqkF</b>    | 34695                   | 5.30      | 535                         | 100                                | 14  |
| <b>YthP</b>    | 26490                   | 5.39      | 195                         | 100                                | 5   |
| <b>YvIB</b>    | 41056                   | 5.50      | 156                         | 100                                | 10  |
| <b>YvyD</b>    | 21966                   | 5.36      | 393                         | 100                                | 11  |
| <b>YwrO</b>    | 19942                   | 5.33      | 166                         | 100                                | 7   |
| <b>YxaB</b>    | 39738                   | 4.72      | 452                         | 100                                | 18  |

61

62 **Supplementary Table 4: Strains used in this study**

| Strain                        | Relevant Genotype                                  | Induction | MIC | Source    |
|-------------------------------|--|-----------|-----|-----------|
| <i>B. subtilis</i> 168        | wild type  | -         | 6*  | 2         |
| <i>B. subtilis</i> 168/DSM402 | wild type  | -         | 8#  | 3         |
| <i>B. subtilis</i> KS69       | <i>amyE::spc Pxyl-msfgfp-mreB</i>                  | 0.3% xyl  | nd  | this work |
| <i>B. subtilis</i> KS70       | <i>amyE::spc Pxyl-msfgfp-mbl</i>                   | 0.3% xyl  | nd  | this work |
| <i>B. subtilis</i> TNVS175    | <i>amyE::spc Pxyl-murG-msfgfp</i>                  | 0.05% xyl | nd  | 4         |
| <i>B. subtilis</i> KS19       | <i>lytABC::neo lytD::tet lytE::cat lytF::spc</i>   | -         | -   | this work |
| <i>B. subtilis</i> L16648     | <i>lytABC::neo lytD::tet lytE::cat lytF::spc</i>   | -         | nd  | 5         |
| <i>B. subtilis</i> HB5343     | <i>psd::ery</i>                                    | -         | 6*  | 6         |
| <i>B. subtilis</i> SDB206     | <i>ywiE::neo clsB::spc clsA::ery</i>               | -         | 6*  | 7         |
| <i>B. subtilis</i> BS23       | <i>cat atpA-gfp:Pxyl-atpA</i>                      | 0.5% xyl  | nd  | 8         |
| <i>B. subtilis</i> HS41       | <i>amyE::tet Pxyl-yhaP-gfp</i>                     | 1% xyl    | nd  | 9         |
| <i>B. subtilis</i> HS64       | <i>amyE::spc Pxyl-WALP23-gfp</i>                   | 1% xyl    | nd  | this work |
| <i>B. subtilis</i> KR318      | <i>amyE::psc Phyperspank -spoVM-gfp</i>            | 1 mM IPTG | nd  | 10        |
| <i>B. subtilis</i> HS65       | <i>amyE::spc Pxyl-gfp-junLZ-MTS<sub>MinD</sub></i> | 1% xyl    | nd  | this work |
| <i>B. subtilis</i> HS208      | <i>amyE::spc Pxyl-MTS<sub>SepE</sub>-junLZ-gfp</i> | 1% xyl    | nd  | 11        |

63 \*determined in LB, #determined in BMM

64

65 **Supplementary Table 5: Oligonucleotides**

| Oligonucleotide | Sequence  |
|-----------------|---|
| JunLZ-for       | gcgcgGGATCCTTGGTGGTCGTATCGCTCGTCC               |
| MTS-rev         | gcgcgAAGCTTAAGATCTTACTCCGAAAAATGA               |
| pSG1154-for     | ATGAGTAAAGGAGAAGAAGACTTTTCAC                    |
| pSG154-rev      | CATCCTAGGAATCTCCTTTCTAG                         |
| WALP23-for      | ATGGCTTGGTGGTTAGCCTTGGCATTGGCGCTTGCTCTCGCCCTGGC |
| WALP23-rev      | CGCCACACAGGGCTAACGCCAATGCCAGGGCGAGAGCAAGCGCC    |
| WALP23-IFfor    | GAGATTCCTAGGATGGCTTGGTGGTTAGCCTTG               |
| WALP23-IFrev    | TCTCCTTACTCATTCTGAGCCGCTTCTGACGCCACACAGGGCTAAC  |

66

67 **Supplementary Movie 1: cWFW-dependent cell lysis**

68 Growth and lysis of *B. subtilis* 168 at 37 °C in solid LB medium in the absence and presence

69 of cWFW (25 μM). Strain used: *B. subtilis* 168 (wild type).

70 SUPPLEMENTARY INFORMATION REFERENCES

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