

Supplementary data

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3 **TABLE S1** Primers used in this work.

| Deleted strain | Primers for the regions of homology (5'-3') ^a | Primers to confirm the deletion (5'-3') |
|----------------|--|---|
| D2 | D2-1F: <u>TTCTAGAGA</u> AAGGTGCCGCTGATGG | KO2F: CGGCGTCATGTGGTA |
| | D2-1R: TT <u>GATCCC</u> ACGCTGGACGAACC | KO2R: GCAGGATCGGGCAAGC |
| | D2-2F: TT <u>GATCCC</u> GTGCTGCGTTGAAGC | |
| | D2-2R: TT <u>GTACCC</u> GGCGGTTTCCCTACC | |
| D5 | D5-1F: TT <u>GTACCT</u> CGTGGTGATGAAGGCGG | KO5F: CGGTGATGCGTGCAATGG |
| | D5-1R: TT <u>GATCC</u> GGCTTTCCTCGATGATGC | KO5R: TTAGGTGCAATGGACGCA |
| | D5-2F: TT <u>GATCC</u> GTCGATGGCGTTCCTGCT | |
| | D5-2R: <u>TTCTAGAC</u> GATATGGTTCATACGCC | |
| D6-7 | D6-1F: TT <u>GTACCT</u> GTAATCGTTTCGTCATCGCC | KO6F: GCACCAGTGCCCGCCGAAT |
| | D6-1R: TT <u>GATCCC</u> CTTGCGCTGAACTGCCTGC | KO6R: CGGACGACCAAAGCGACGC |
| | D6-2F: TT <u>GATCC</u> TGACGCTCTATACGA | |
| | D6-2R: <u>TTCTAGAG</u> CGATCGGCGTCGCGCTCGA | |
| D8 | D8-1F: <u>TTCTAGAC</u> GACGATGGGCGTGTT | KO8F: GCTTGACGATGTAGT |

D8-1R: TTGGATCCGGACGGCTGGAGTTGCT

KO8R: CGGCTCGGTTTTGACG

D8-2F: TTGGATCCGGCAGGTCCTCCGTCA

D8-2R: TTGGTACCCCGTCCGTCTGTCCG

D10

D10-1F: TTTCTAGACGCTACTGGCTGTCCG

KO10F: AGGAACATGGCGAGAAT

D10-1R: TTGGATCCGTTGCAGGCGAACCA

KO10R: CCGAGAGAAGATGGCG

D10-2F: TTGGATCCGTTGCCCGCTGCTGAG

D10-2R: TTGGTACCCACTCCTAGAGATGTC

D11

D11-1F: TTTCTAGACTGCTGGCGGGGTAAA

KO11F: AGCAGTTGTCAAGGCA

D11-1R: TTGGATCCATTACGAAGTCACTCA

KO11R: TTGAAGATCACGGTG

D11-2F: TTGGATCCCGCCGTCGGTATTCG

D11-2R: TTGGTACCGCCATCACGGTCTCG

D12

D12-1F: TTGGTACCCGCCATCCGCCGATCACG

KO12F: GACATGAGCGATTGCGATTC

D12-1R: TTGGATCCCGAGCACGGACGCCTG

KO12R: CTTGACCTTGTCGCCCGCAT

D12-2F: TTGGATCCTCGCCGTCGTGCTCGCC

D12-2R: TTTCTAGATTTCGCCTTGAACGCCATC

D13

D13-1F: TTGGTACCTGGTCCCTGCTCTGCTC

KO13F: TCATAAAGATGCGATGCGC

D13-1R: TTGGATCCGGCGCTCGATGATCTTG

KO13R: GATTGCGTGATAGTGATGAA

D13-2F: TTGGATCCTCGACCAGTACAAGCAGGCCG

D13-2R: TTTCTAGAGGTCAGCCCGCTGCGTTT

D14

D14-1F: TTTCTAGAAAGTTGAGTCCATTCTG

KO14F: ACTGCACGAATCAGGTT

D14-1R: TTGGATCCAGGGCATCGTGAAAAA

KO14R: GCCGCTGAACAACAT

D14-2F: TTGGATCCCAGGTAAGCGTAAGC

D14-2R: TTGGTACCATCCGAGTTTCGTCAC

D15

D15-1F: TTTCTAGACGAGAACGGCTTGGT

KO15F: CGTTCGCATTGCTGTC

D15-1R: TTGGATCCACAGTTGCTGACGAG

KO15R: CGTGATTCATCAGTTG

D15-2F: TTGGATCCCGTTGTAATTCCAGAT

D15-2R: TTGGTACCTCGCAGGAGGAACTCG

D16

D16-1F: TTGGTACCTCATGGATTCCTCTTCT

KO16F: CGACACGAGCAAGGCCGTCA

D16-1R: TTGGATCCGAAAGCTGCGGAACGACT

KO16R:TCGGCTTGCGTTGCGCTG

D16-2F: TTGGATCCCGAAACGAGATCTGGTTCG

D16-2R: TTTCTAGAGGCGAACATCAAGGTTT

4 ^aRestriction endonuclease sites incorporated in the oligonucleotide sequences are underlined

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TABLE S2 Primers used to study the gene expression

| Gene number | FW primer | RV primer |
|--------------------|-----------------------|----------------------|
| BCAL1675 (D3) | GCGATCGTCGGGATCACCGT | GGCCGAGAAGCCGATCGACA |
| BCAL2820 (D4) | CTGCCATCGGCTGGCGCGAA | GCACCGATACGCGCAGGTCT |
| BCAM0925 (D8) | CGCTTCAGCAGCAACCGGTGA | GGCCGACGGAACAGCAGCT |
| BCAM0926 (D8) | GACCGCGGTATTCCTGCCGA | ACGGCCATCGACAGCGTGAA |
| BCAM1945 (D9) | TCCGACGCTGACGCAGTTGA | GTAGCGCGATACGGCGACCT |

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9 **TABLE S3** Average number of CFUs recovered from treated and untreated *B. cenocepacia* biofilms.
 10 (n ≥ 6 for each condition).

| | Untreated | CIP (32 µg/ml) ^a | TOB (1024 µg/ml) |
|---------------|------------------------|-----------------------------|------------------------|
| 12 J2315 (WT) | 1.75 x 10 ⁸ | 9.31 x 10 ⁵ | 4.94 x 10 ⁴ |
| 13 D1 | 8.44 x 10 ⁷ | 3.93 x 10 ⁶ | 4.57 x 10 ³ |
| 14 D2 | 9.68 x 10 ⁷ | 6.69 x 10 ⁶ | 5.19 x 10 ³ |
| 15 D3 | 8.50 x 10 ⁷ | 6.03 x 10 ⁴ | NR ^b |
| 16 D4 | 1.52 x 10 ⁸ | 3.97 x 10 ⁵ | 8.62 x 10 ⁴ |
| 17 D5 | 4.65 x 10 ⁷ | 6.37 x 10 ⁴ | 8.15 x 10 ² |
| 18 D6-7 | 1.64 x 10 ⁸ | 1.46 x 10 ⁶ | 4.57 x 10 ⁴ |
| 19 D8 | 8.93 x 10 ⁷ | 8.42 x 10 ⁵ | 1.67 x 10 ¹ |
| 20 D9 | 7.01 x 10 ⁷ | 2.05 x 10 ⁶ | 2.00 x 10 ¹ |
| 21 D10 | 9.25 x 10 ⁷ | 2.60 x 10 ⁶ | 3.66 x 10 ⁴ |
| 22 D11 | 6.13 x 10 ⁷ | 1.63 x 10 ⁶ | 1.86 x 10 ⁴ |
| 23 D12 | 6.15 x 10 ⁷ | 1.48 x 10 ⁵ | 1.26 x 10 ⁴ |
| 24 D13 | 8.54 x 10 ⁷ | 2.09 x 10 ⁶ | 1.90 x 10 ⁵ |
| 25 D14 | 1.19 x 10 ⁸ | 2.73 x 10 ⁶ | 2.04 x 10 ⁵ |
| 26 D15 | 1.62 x 10 ⁸ | 1.20 x 10 ⁶ | 7.14 x 10 ³ |
| 27 D16 | 7.76 x 10 ⁷ | 7.98 x 10 ⁴ | 2.68 x 10 ⁴ |

28 ^aCIP, ciprofloxacin; TOB, tobramycin

29 ^bNR, no cells recovered

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33 **FIGURE S1.** Average absorbances (at 590 nm) obtained with the crystal violet assay on biofilms for
34 the WT strain and strains D3 and D4. Error bars indicate standard deviations of 10 replicates.

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