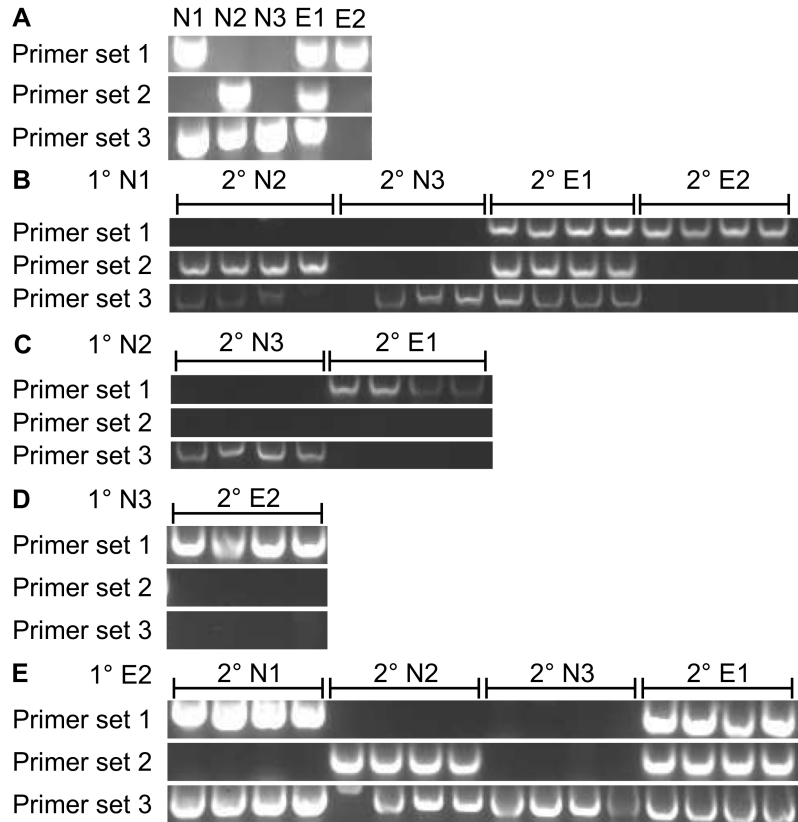


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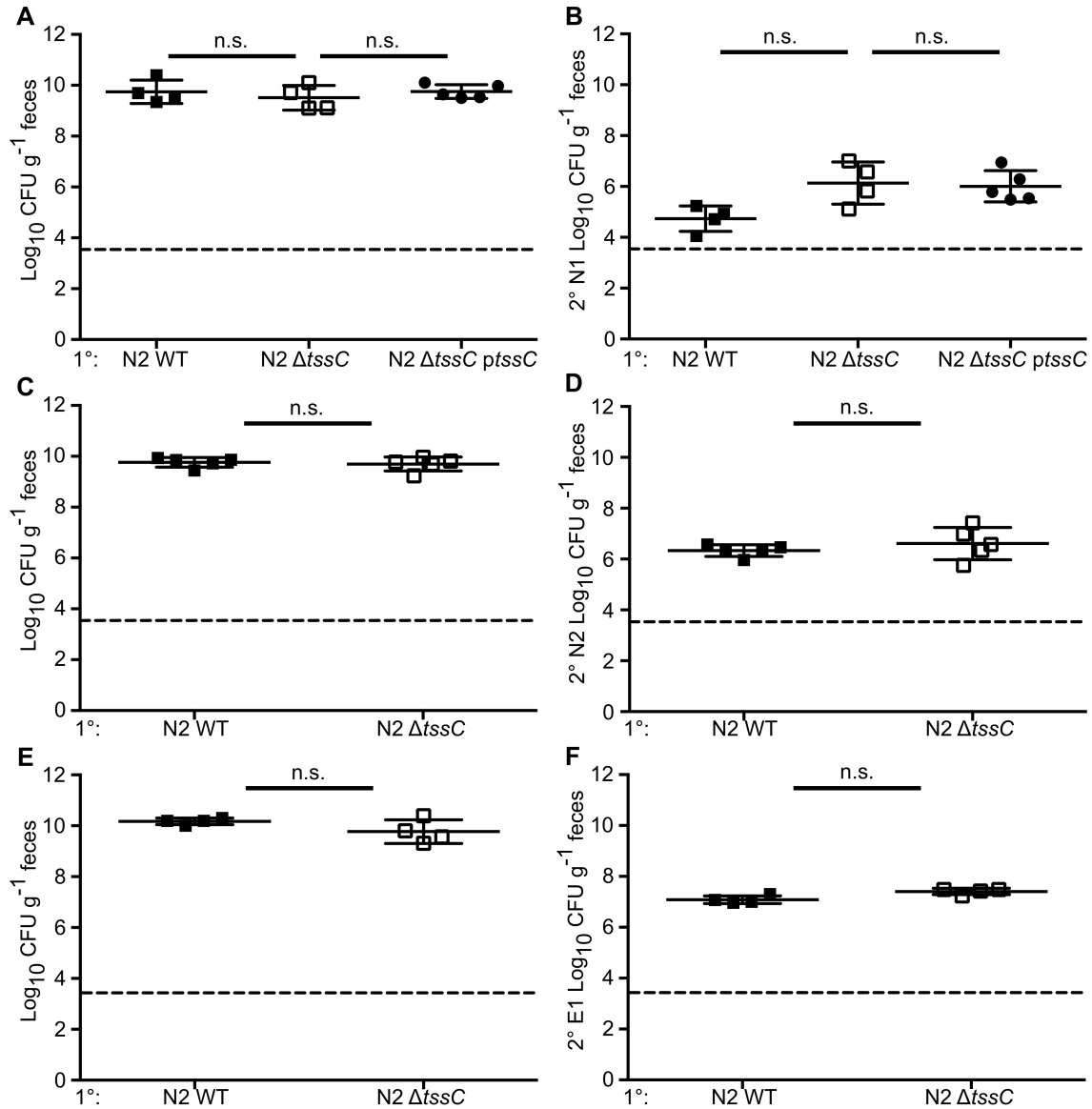
**Appendix Figure S1.**

**Successful secondary challenge strain identities are confirmed via colony PCR.**

A. Colony PCR with three primer sets to distinguish the strains was performed on the five *B. fragilis* strains in this study.

B-E. Colony PCR was performed on the successful secondary challenge strains four weeks post-challenge.

Data information: Each column of lanes represents a colony from each mouse in the indicated groups (n=4).

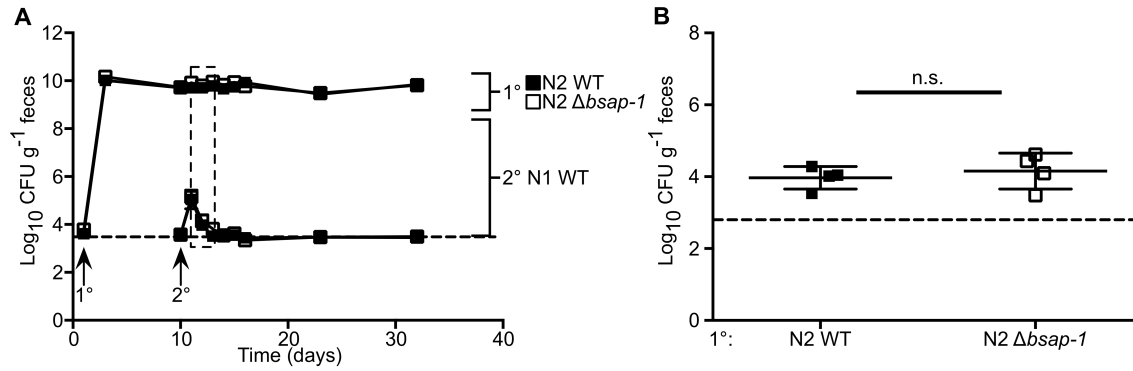


**Appendix Figure S2.**

**T6S has no effect on either primary colonization of N2 or secondary challenge strain one-day post-secondary challenge.**

A-F. Primary colonization of SPF mice with N2 WT, T6SS mutant ( $\Delta tssC$ ) and complemented ( $\Delta tssC$  pTssC) followed by secondary challenge with N1 WT (A and B; n=5 mice) N2 WT (C and D; n=5) or E1 WT (E and F; n=4) was performed. Fecal CFU for primary (A, C and E) and secondary strains (B, D and F) was determined one-day post-secondary challenge.

Data information: Results are representative of three independent experiments. Error bars: mean  $\pm$  SD. A dashed line denotes limit of detection. n.s., not significant. Statistical significance was determined by unpaired, parametric, two-tailed Student's  $t$ -test (C-F) or one-way ANOVA, Tukey's multiple comparisons test (A and B).



**Appendix Figure S3.**

**BSAP-1 has no effect on N2 colonization resistance against N1.**

A and B. Fecal CFU was determined after primary colonization with N2 WT or N2 *bsap-1* mutant ( $\Delta$ *bsap-1*) followed by secondary challenge with N1 WT for four weeks-post secondary challenge (A; n=4 mice per group). Secondary challenge fecal CFU was compared two days post-challenge between N2 WT and  $\Delta$ *bsap-1* groups (B). A dashed line denotes limit of detection.

Data information: Results representative of two independent experiments. Error bars: mean  $\pm$  SEM (A), mean  $\pm$  SD (B). n.s., not significant. Statistical significance was determined by unpaired, parametric, two-tailed Student's *t*-test.

**Appendix Table S1. Strain names, abbreviations and inherent antibiotic resistances.**

| Strain abbreviation        | Reference name          | Tetracycline | Rifampicin | Chloramphenicol |
|----------------------------|-------------------------|--------------|------------|-----------------|
| N1                         | NCTC 9343<br>ATCC 25285 | Sensitive    | Sensitive  | Sensitive       |
| N2                         | TM4000<br>638R          | Sensitive    | Resistant  | Sensitive       |
| N3                         | YCH46                   | Resistant    | Sensitive  | Sensitive       |
| E1                         | ATCC 43858              | Sensitive    | Sensitive  | Sensitive       |
| E2                         | ATCC 43859              | Resistant    | Sensitive  | Sensitive       |
| <i>B. thetaiotaomicron</i> | ATCC 29148              | -            | Sensitive  | Sensitive       |
| <i>B. vulgatus</i>         | ATCC 8482               | -            | Sensitive  | Sensitive       |

**Appendix Table S2. Primers.**

| Purpose                        | Direction | Sequence   |
|--------------------------------|-----------|--|
| Generation of pAH1             | F         | ATAGCGTCTAGATTTTCTTGGGCATACTGCGTT                        |
|                                | R         | ATAGCGTCAGACATCTTACACCAAAATTGCACA                        |
| Validate pAH1                  | F         | CGCGGTGGCGGCCGCT   |
|                                | R         | AAGGAGCAATTTAAATTTAAAATAG                                |
| Generation of pAH2             | F         | GCGGCCGCGAATTCGATAACAGCCGGTG                             |
|                                | R         | GCGGCCGCGCTCCCCGACCGATGAT                                |
| Generation of pAH2-CAT         | F         | GGTACCAAAGATCTGAAAGAGAGACAATG                            |
|                                | R         | GGTACCCGAATTTCTGCCATTCATCCG                              |
| <i>TssC</i> mutant construct   | US F      | GGTACCGGTATTCGTCCACCGTAGTG                               |
|                                | US R      | AGACAATAAGAACTATGGAAGATCAGGCGTAATAAGAAT<br>AAAATATG      |
|                                | DS F      | CATATTTTATTCTTATTACGCCTGATCTTCCATAGTTCTTA<br>TTGTCT      |
|                                | DS R      | GGTACCTCTTGTAGTCTGTATTAACAAAT                            |
| <i>Bsap-1</i> mutant construct | US F      | GGATCCATAAGGAGTTGAAAGATATGAATC                           |
|                                | US R      | ATAATTTCAAGAGGTTATGGTCTGAATAGCATTAGTAAAA<br>GTCCAA       |
|                                | DS F      | TTGGACTTTTACTAATGCTATTCAGACCATAACCTCTTGA<br>AATTAT       |
|                                | DS R      | GGATCCAAAAGATTCAGCCACAGCAATG                             |
| <i>Bte2</i> mutant construct   | US F      | GGATCCTGCATCAATACTGAAGAGAATG                             |
|                                | US R      | TAAGAGAATGTTTATGACCTTTCGTCATCTTTCATCTTCT<br>CATTTT       |
|                                | DS F      | AAAATGAGAAGATGAAAGATGACGAAAGGTCATAAACAT<br>TCTCTTA       |
|                                | DS R      | GGTACCCTCTTGTATCTGTTTGAAAGCT                             |
| Generation of pTssC            | US F      | GGTACCCTTTTTTTATATTTAATATGAATTTAAT                       |
|                                | US R      | TAAAACCCTTAAAAGTTTTAAACAAAATGGAAGATAATAAAA<br>AATAAGTCTG |
|                                | DS F      | CAGACTTATTTTTATTATCTTCCATTTTGTTTAAACTTTTA<br>AGGGTTTTA   |
|                                | DS R      | GGTACCTTACGCCTGTTTCGTAATTTGTA                            |
| Generation of pBTI2a           | US F      | GGTACCCTTTTTTTATATTTAATATGAATTTAAT                       |
|                                | US R      | AGAGAATGTTTATGACCTTTCATTTTGTTTAAACTTTTAAG<br>GGTTTTA     |
|                                | DS F      | TAAAACCCTTAAAAGTTTTAAACAAAATGAAAGGTCATAAAA<br>CATTCTCT   |
|                                | DS R      | GGTACCTTATTGCTTTTCAGCCTCTTTC                             |

**Appendix Table S2. Continued.**

| Purpose                                     | Direction | Sequence                 |
|---|-----------|--------------------------|
| <i>B. fragilis</i><br>genotyping<br>primers | Set 1 F   | CCTAGATTGAATCATATTCTATAC |
|   | Set 1 R   | AATCTCAAACCTTGAAGGCTG    |
|   | Set 2 F   | GAGTGCAGTAGTTTTGATAGT    |
|   | Set 2 R   | CAAGCGACTGCTCCAAAAGC     |
|   | Set 3 F   | ACGCCCTATTAAAGTGATTATTT  |
|   | Set 3 R   | TCAGCCCTGCTCTCCGATT      |
| BFT qRT-PCR                                 | F         | AAGGGCTGGATGGCTTTACT     |
|   | R         | GGGATACATCAGCTGGGTTG     |
| 16S qRT-PCR                                 | F         | CAGTCTTGAGTACAGTAGAGGTGG |
|   | R         | GTGGACTACCAGGGTATCTAATCC |