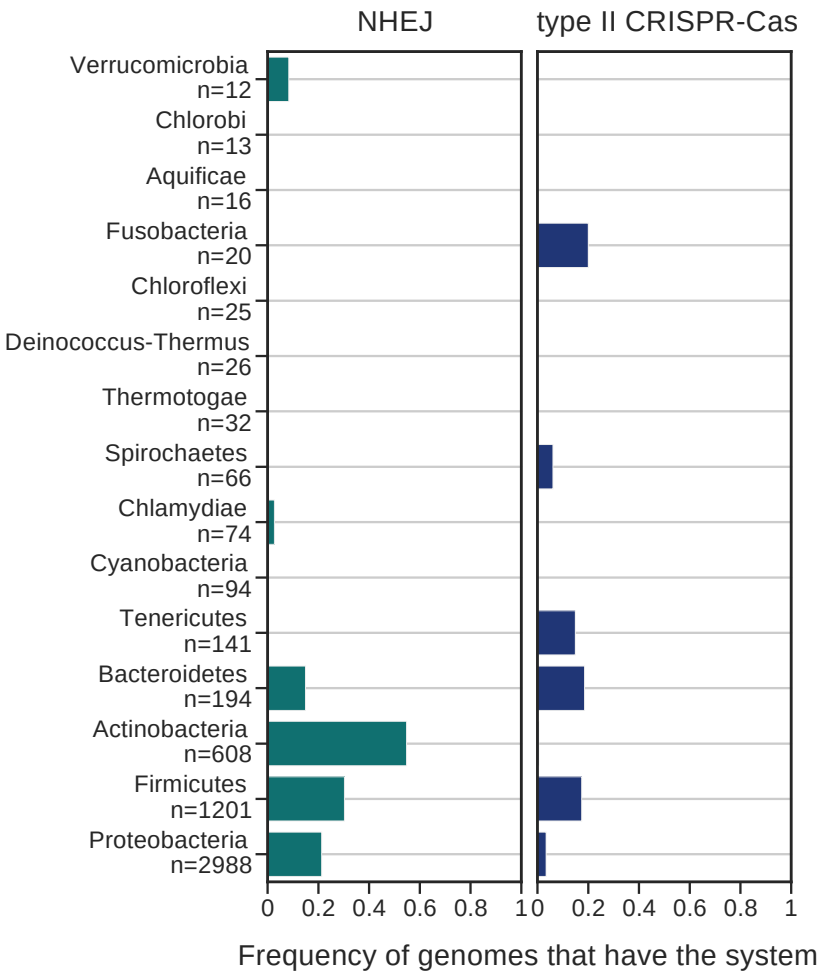
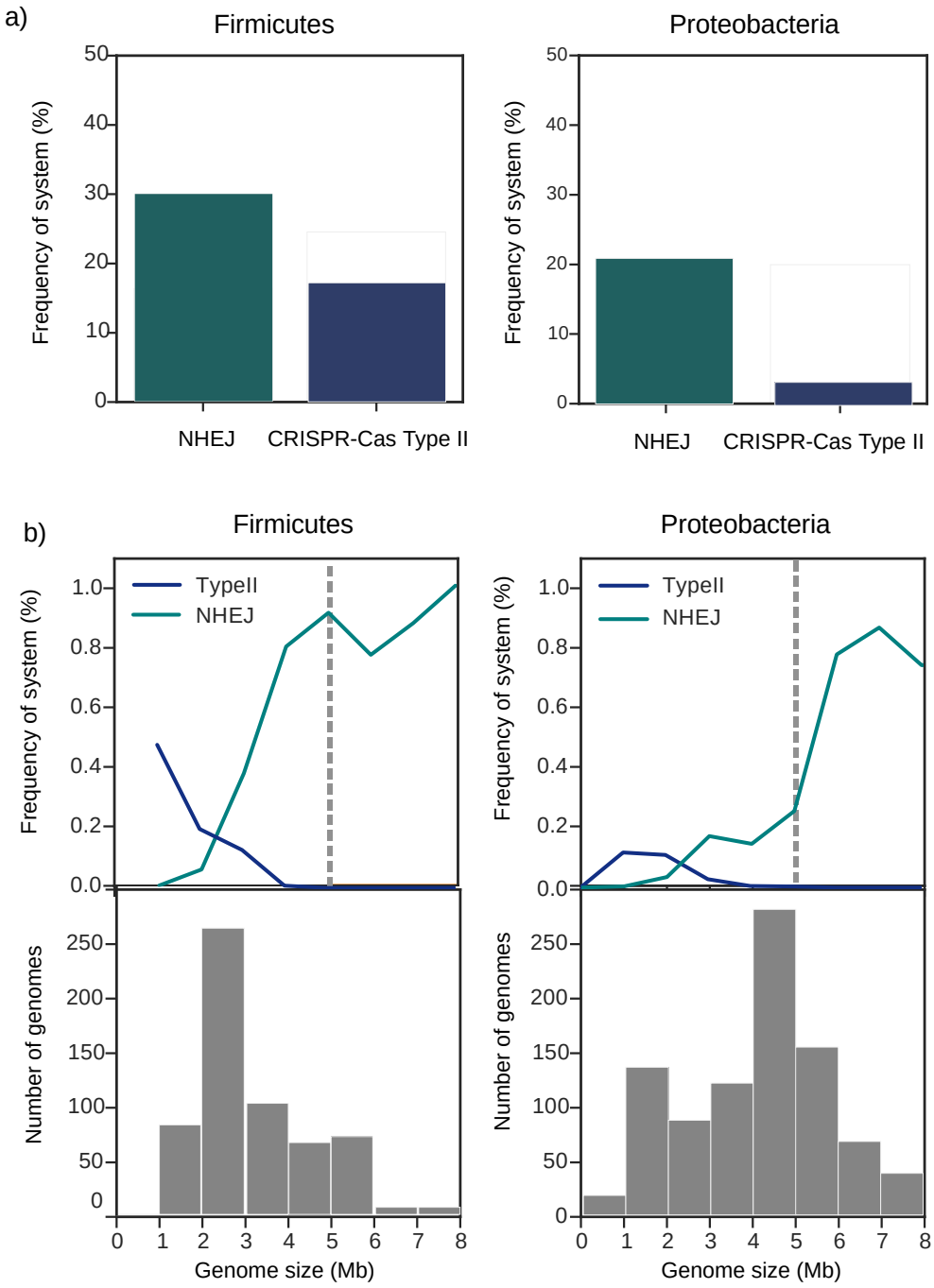
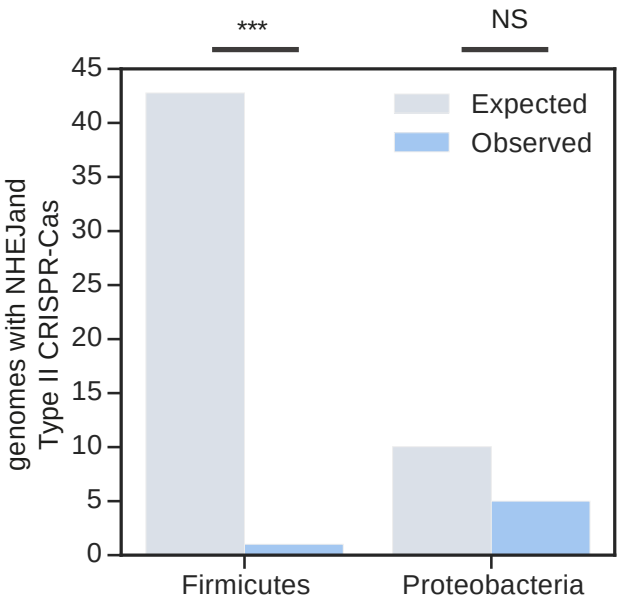
# Supplementary Figures



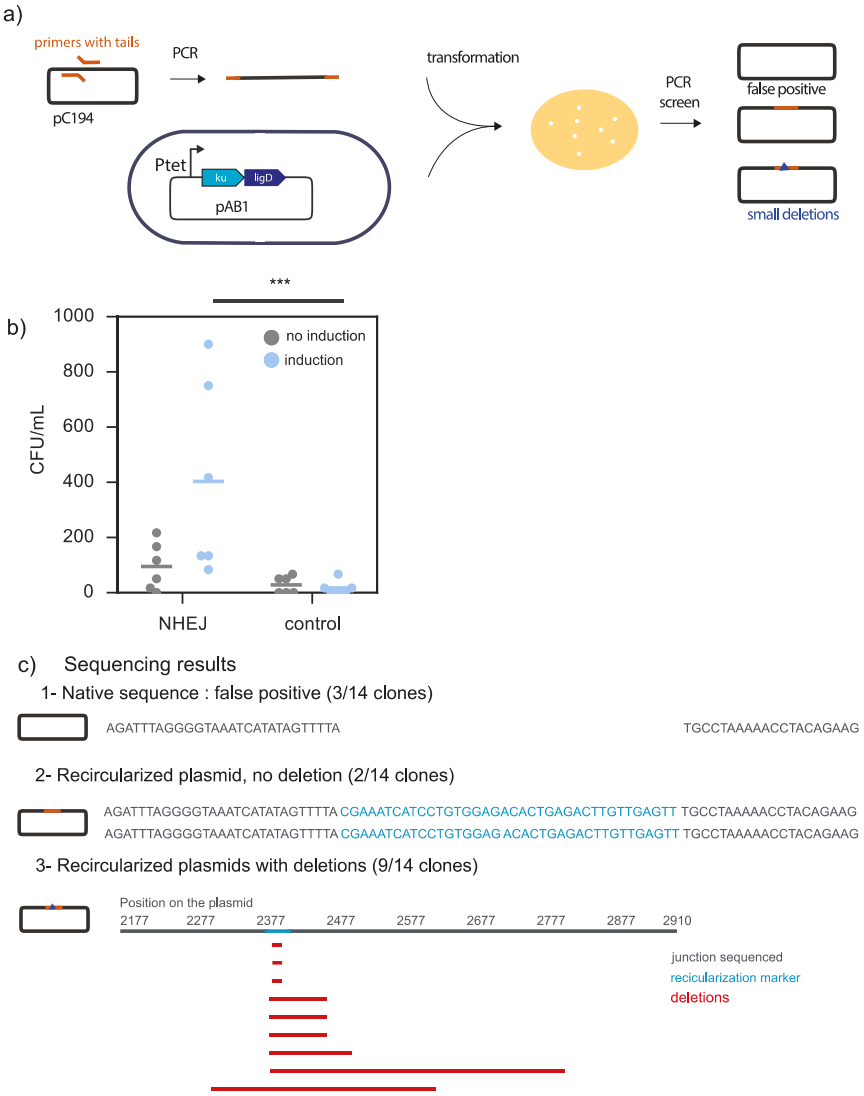

**Supplementary Figure 1: Frequency of NHEJ and CRISPR-Cas systems by clades.**



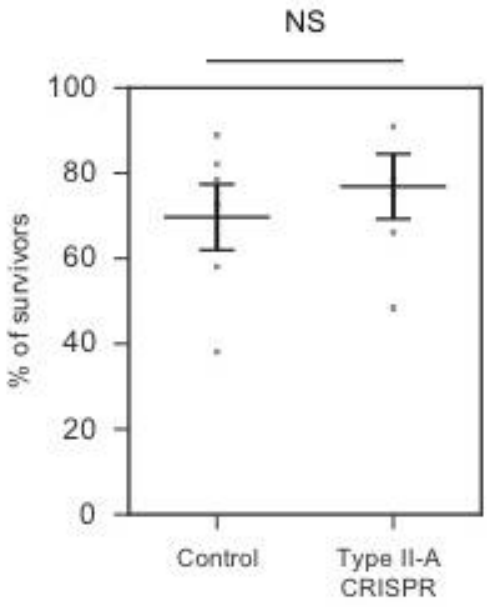

**Supplementary Figure 2: Distribution of NHEJ and CRISPR-Cas systems in the genomes of Firmicutes and Proteobacteria. a,** Frequency of NHEJ and CRISPR-Cas systems in the 1201 Firmicutes and 2988 Proteobacteria genomes. **b,** Frequency of NHEJ and CRISPR-Cas systems in function of genome size. The histogram on the bottom represents the distribution of genome sizes in each clade. The frequency represents the frequency of genomes carrying a system within the genome size range. Vertical line corresponds to the size cut-off (5Mb).




**Supplementary Figure 3: Associations between NHEJ and Type II CRISPR-Cas systems in Firmicutes and Proteobacteria genomes**. The expected values are the product of the marginal row and column totals divided by the grand total of the contingency tables. Statistics were calculated using Fisher's Exact Test. ** P< 0.05, *** P< 0.01

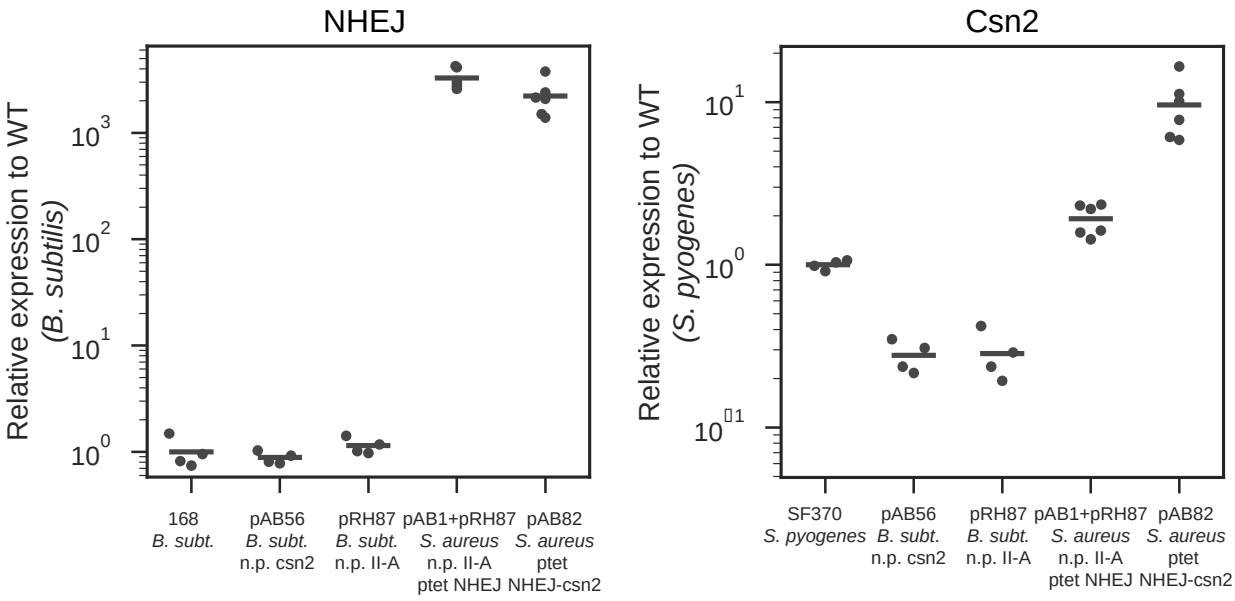



**Supplementary Figure 4: NHEJ system of *B. subtilis* is functional in *S. aureus*. a,** Introduction of a linearized plasmid providing resistance to chloramphenicol (pC194) into cells carrying the NHEJ system (pAB1) either in the presence or absence of aTc (induction) in *S. aureus*. Only cells that re-circularized the plasmid could form colonies on chloramphenicol plates. **b,** The CFU of five replicates is represented, error bars corresponds to s.e.m. *** P< 0.01 Wilcoxon test **c,** Sequencing results of transformants recovered after NHEJ induction. The primers used to linearize pC194 were designed to carry 5' tails (in blue). If plasmids were recircularized by the NHEJ system, we expected to find those tails sequence at the junction. Results are shown for clones recovered after NHEJ induction. Blue corresponds to overhangs and red to deletions. The insertion of the intact tail sequences at the junction was observed in 2/14 colonies recovered after NHEJ induction. In 9/14 colonies, small deletions were observed. The remaining 3/14 colonies were false positives. In contrast, all colonies obtained in the control experiment corresponded to the pC194 plasmid that was likely present in trace amounts in the PCR products used for the transformation.




**Supplementary Figure 5 : Irradiation of *S. aureus* cells**

*S. aureus* cells carrying a type II-A CRISPR-Cas systems or a control plasmid were irradiated. The survival rate was determined as the ration of CFUs obtained for irradiated over non-irradiated cells. No significant difference was observed (n=5 Wilcoxon, P=0.5).




**Supplementary Figure 6 : Expression level of NHEJ and Csn2**

Expression of NHEJ and Csn2 was measure using q-PCR in strains used in the study (see Supplementary Table 3 for a description of the plasmids). Expression was normalized to the 16s_rRNA expression in each strain measured using the same set of primers. Expression is shown relative to the wild-type: *B. subtilis* 168 for NHEJ and *S. pyogenes* SF370 for Csn2.

# Supplementary Tables

**Supplementary Table 1: Detected systems and their frequencies by genomes**

| Systems | Bacterial Genomes (2482) | Frequency (%) (Bacteria) |
|---|---|---|
| Type I CRISPR-Cas | 1584 | 28.5 |
| Type II CRISPR-Cas | 384 | 6.9 |
| Type III CRISPR-Cas | 306 | 5.5 |
| NHEJ | 1376 | 24.7 |

**Supplementary Table 2: Plasmids used in this study**

| **Plasmids from other studies** | | **Described in** |
|---|---|---|
| pC194/pE194 | Replicative plasmid in *S. aureus*, respectively CmR, ErmR, | [1,2] |
| pT181 | Replicative plasmid in *S. aureus*, tetR | [3] |
| pMUTIN4 | Integrative plasmid in *B. subtilis*, ErmR | [4] |
| pNZ123 | Replicative plasmid in *S. thermophilus*, CmR | [5] |
| pLZ12 | Replicative plasmid in *S. aureus* and *E. coli* | [6] |
| pCN57 | Plasmid with a working GFP in *S. aureus* | [7] |
| pRH87 | pC194 with type II-A CRISPR-Cas system from *S. pyogenes* with one spacer in the CRISPR array | [8] |
| pRH61,pRH62,pRH63 | same as pRH87 but without respectively *cas1*,*cas2*, *csn2* | [8] |
| pRH121 | same as pRH87 but with *dcas9* instead of *cas9* | [8] |
| pDB114 | pC194 with *cas9* and reprogrammable spacer | [9] |
| pWJ153 | pE194 Ptet inducible target vector | [9] |

| Plasmids created for this study | | Insert (Primer ; Template) | | Vector (Primer ; Template) | | Cloned in |
|---|---|---|---|---|---|---|
| pAB1 | pE194 with NHEJ system from *B. subtilis* under tet promoter (Ptet) | AB+AB4 | *B. subtilis* genomic DNA | AB1+AB2 | pWJ153 | *S. aureus* |
| pAB2 | pT181 with a protospacer from PhiNM4 phage | | | AB9+AB10 | pT181 | *S. aureus* |
| pAB12 | pMUTIN with ykoV homologies (to build ykoV mutant in *B. subtilis*) | AB69+AB70 | *B. subtilis* genomic DNA | AB67+AB68 | pMUTIN 4 | *E. coli* |
| pAB13 | pMUTIN with ykoU homologies (to build ykoU mutant in *B. subtilis*) | AB73+AB74 | *B. subtilis* genomic DNA | AB71+AB72 | pMUTIN 4 | *E. coli* |
| pAB17 | pE194 with ligD under tet promoter (Ptet) | | | AB85+AB86 | pAB1 | *S. aureus* |
| pAB18 | pE194 with Ku under tet promoter (Ptet) | | | AB87+AB88 | pAB1 | *S. aureus* |
| pAB23 | pLZ12 with NHEJ under tet promoter (Ptet) | AB95+AB96 | pAB1 | AB97+AB98 | pLZ12 | *E. coli* |
| pAB24 | pLZ12 with Ku under tet promoter (Ptet) | AB95+AB96 | pAB18 | AB97+AB98 | pLZ12 | *E. coli* |
| pAB25 | pLZ12 with ligD under tet promoter (Ptet) | AB95+AB96 | pAB17 | AB97+AB98 | pLZ12 | *E. coli* |
| pAB62 | pLZ12 with GFP under tet promoter (Ptet) | ACV69+ACV70 | pCN57 | ACV64+ACV65 ACV67+ACV68 | pAB23 | *E. coli* |
| pAB66 | pNZ123 with NHEJ under P8 constitutive *S. thermophilus* promoter | AB244+AB245 AB246+AB247 | pAB1 ; *S. thermophilus* DNA | AB242+AB243 | pNZ123 | *E. coli* |
| pAB69 | pNZ123 with GFP under P8 constitutive *S. thermophilus* promoter | AB50+AB251 AB252+AB253 | pCN57 ; *S. thermophilus* DNA | AB248+AB249 | pNZ123 | *E. coli* |
| pAB56 | pC194 with csn2 under type II-A natural promoter | Reciculrized | | AB224+AB225 | pRH87 | *S. aureus* |
| pAB81 | pAB1 with csn2 cloned directly after ligD | AB280+AB281 | pAB56 | AB278+AB279 | pAB1 | *S. aureus* |
| pAB82 | pAB1 with csn2 cloned with the promoter of the cas operon of *S. pyogenes* | AB284+AB285 | pAB56 | AB282+AB283 | pAB1 | *S. aureus* |
| pMD021 | pC194 with *cas9* and spacer targeting PhiNM4 | D035+D036 | Hybridized primers | Digested with Bsa1 | pDB114 | *S. aureus* |

**Supplementary Table 3: Primers used in the study**

| | |
|---|---|
| AB1 | GCTCTTTCCGTGGTTCATATTTATCAGAGCTCGTGCTATAAT |
| AB2 | GAGTACGATTCATTTGATATGCCTCCTCTAGGTCATTTGATATGCCTCCG |
| AB3 | CAAATGACCTAGAGGAGGCATATCAAATGAATCGTACTCCTTCTCTTCAC |
| AB4 | GATAAATATGAACCACGGAAAGAGCTGACTTCATTAG |
| AB9 | AAAAATGTTTTAACACCTATTAACGTAGTATGGAGTGGCTAGCATTTTGC |
| AB10 | CCATACTACGTTAATAGGTGTTAAAACATTTTTAAACTGCTTTTCAGAAC |
| AB67 | AGTCCCAGGTGTACCTGATCAGAAAACCGCCTCGCG |
| AB68 | ACAAAGTCAACAATCTCAACGATTCTCCGTGGGAACAAACGG |
| AB69 | GTTTGTTCCCACGGAGAATCGTTGAGATTGTTGACTTTGTTCAGCTTCAG |
| AB70 | TCACCGCGAGGCGGTTTTCTGATCAGGTACACCTGGGACTTGAG |
| AB71 | ACTATGCGCCGGATTTTGTTAGAAAACCGCCTCGC |
| AB72 | TGTTAAGTATTGAACAGCTGGATTCTCCGTGGGAACAAACG |
| AB73 | GTTTGTTCCCACGGAGAATCCAGCTGTTCAATACTTAACAATTCTCCAAG |
| AB74 | TCACCGCGAGGCGGTTTTCTAACAAAATCCGGCGCATAGTCC |
| AB85 | CGCATGGCGTTTACCATGCA |
| AB86 | GGTCATTTGATATGCCTCCGG |
| AB87 | GAAGTCAGCTCTTTCCGTGGTTC |
| AB88 | GCGCTATGATGTGCCGGAG |
| AB95 | CTAATGAATTCATCTGCAGGAAAGAAATTAGATAAATCTCTCATATCTTTTATTCAATAATCGCAT |
| AB96 | GGTCGTCAGACTGATGGGCCAATTATAGCACGAGCTCTGATAAATATGAACCAC |
| AB97 | TCAGAGCTCGTGCTATAATTGGCCCATCAGTCTGACGAC |
| AB98 | GAGATTTATCTAATTTCTTTCCTGCAGATGAATTCATTAGGATCCAGA |
| AB224 | ATTTACATGGTGAAAGAAATAATTGTATTGCAAACTCC |
| AB225 | TTGCCTCCTAAAATAAAAAGTTTAAATTAAATCC |
| AB242 | ACTTCGAACTAGCAATACTGCTCTCTAGAGAATTCAGTACTGGATCT |
| AB243 | TTCCGTGGTTCATATTTATCCTCAAGCTTCTCGAGTGCATATTTTCG |
| AB244 | CTCGTGCGAGGTTTTTACATATGAATCGTACTCCTTCTCTTCACACTAAAG |
| AB245 | ATGCACTCGAGAAGCTTGAGGATAAATATGAACCACGGAAAGAGCTGAC |
| AB246 | GTACTGAATTCTCTAGAGAGCAGTATTGCTAGTTCGAAGTCATCCTTTTTTATAGG |
| AB247 | AGAGAAGGAGTACGATTCATATGTAAAAACCTCGCACGAGTAGTTATTT |
| AB248 | CGAACTAGCAATACTGTAAGAGCTCTCTAGAGAATTCAGTACTGGATCT |
| AB249 | CAAATAAGGCGCGCCTATTCCAAGCTTCTCGAGTGCATATTTTCG |
| AB250 | CTCGTGCGAGGTTTTTACATATGAGTAAAGGAGAAGAACTTTTCACTGGAG |
| AB251 | ATATGCACTCGAGAAGCTTGGAATAGGCGCGCCTTATTTGTATAGTT |
| AB252 | ACTGAATTCTCTAGAGAGCTCTTACAGTATTGCTAGTTCGAAGTCATCCT |
| AB253 | AGTTCTTCTCCTTTACTCATATGTAAAAACCTCGCACGAGTAGTTATTT |
| AB278 | GGTATAATACTCTTAATAAAAAGTCAGCTCTTTCCGTGGT |
| AB279 | GTAAATTTGCCTCCTAAAATCATTAGTCAGCTCTTTTTCTTCAACTGATG |

| | |
|---|---|
| AB280 | AGAAAAAGAGCTGACTAATGATTTTAGGAGGCAAATTTACATGGTGAAAGAA |
| AB281 | ACCACGGAAAGAGCTGACTTTTTATTAAGAGTATTATACCATATTTTTAGTTATTAAG |
| AB282 | AAATGCAGTAATACAGGGGCTCGTGCTATAATTATACTAATTTTATAAGGAGG |
| AB283 | CAAAAAATATTACCCAATACGCTCTGATAAATATGAACCACGGAAAGAG |
| AB284 | TGGTTCATATTTATCAGAGCGTATTGGGTAATATTTTTTGAAGAGATATTTTGAAAAAG |
| AB285 | TTAGTATAATTATAGCACGAGCCCCTGTATTACTGCATTTATTAAGAG |
| ACV64 | ACACATGGCATGGATGAACTATACAAATAATTTCTAAATAAGAATATTTGGAGAGCACCGTTC |
| ACV65 | AGCATAACCTTTTTCCGTGATGGTA |
| ACV66 | TTGATATGCCTCCTCTAGGTCATTTG |
| ACV67 | TATAAATTTAACGATCACTCGTTACCATCACGG |
| ACV68 | TCCAGTGAAAAGTTCTTCTCCTTTACTCATTTGATATGCCTCCTCTAGGTCATTTG |
| ACV69 | ATGAGTAAAGGAGAAGAACTTTTCACTGGA |
| ACV70 | TTATTTGTATAGTTCATCCATGCCATGTGTAAT |
| D035 | AAAAATGTTTTAACACCTATTAACGTAGTATGTTTTAGAGCTATGCTGTTTTGA |
| D036 | ATACTACGTTAATAGGTGTTAAAACATTTTTGTTTTGGGACCATTCAAAACAGC |
| B329 | ACACTGAGACTTGTTGAGTTTGCCTAAAAACCTACAGAAG |
| B330 | CTCCACAGGATGATTTCGTAAAACTATATGATTTACCCCTAAATCT |
| A9 | TCAACGCACAATAAATTTTCTCGGC |
| A10 | TACTTAAAAGAAATTGATCCAACCG |
| AB103 | GCCCTCGAGTTGACAAGGACAGTTATTG |
| AB104 | CAATTCGAATCTTGATTTGCTGTC |

**Supplementary Table 4: Example of spacers acquired during adaptation experiments of *S. aureus* against the phage PhiNM4**

| |
|---|
| AGTTAAAAAGAATTTAAAGTCAAGAAGTA |
| ATGTTGATGGATCGTATCAAAGCGACATAC |
| AGGAATTGAGACACCTCAATATATACTTGC |
| ACACAAAGAAGTACATCAAGGGACAATTAC |
| CGAGCAAAGTTTCATCCGTTTAAATCAATA |
| TTAACGGTATGGAAGAAGCGAGTATCAATA |
| TACCGAATGAATTTTTAAAATATTCAGGCA |
| TCTTAAAGTTATTGAAGAAAGGTTATAACA |
| GGCAATGTTATTTTATCGGATTTTAAAAAC |
| GCTAATGACAGACCATTATTTGATGCTAAC |
| GACAAAATCGAACTATCATTAAAAGTTAAA |
| GCTATAGACGGAAGTTTCAACTTATTATAA |
| ACGACAGATATACGTCAGCGATTTATAATC |
| ACCGAATGAATTTTTAAAACATTCAGGCA |
| CAAATCTATTCAAGATACTATCGAAGCTGT |

**Supplementary Table 5: Primers used for RT-qPCR**

| Targeted genes | Primer name | Sequences (5' to 3') |
|---|---|---|
| Ku | LC1340_Ku_For | GGATCGATCAGCTTCGGATTAG |
| Ku | LC1341_Ku_Rev | TGGTGCGTGATCCTCTTTATG |
| Csn2 | LC1342_csn2_For | GCAAACTCCGATGAAAGACTTG |
| Csn2 | LC1343_csn2_Rev | ACCGCCTCTTAATGGAATCG |
| 16s_rRNA | LC1344_16s_For | AGGCAGCAGTAGGGAATCTT |
| 16s_rRNA | LC1345_16s_Rev | GCTGCTGGCACGTAGTTAG |

# Supplementary Notes

### Supplementary Note 1: Functionality of NHEJ system from *B. subtilis in S. aureus*

**NHEJ system from *B. subtilis* is functional in *S. aureus***

We first tested the functionality of the NHEJ system from *B. subtilis* in *S. aureus*. To do so, we cloned *ku* and *ligD* from *B. subtilis* into the staphylococcal vector pE194 under a tetracycline-inducible promoter Ptet (plasmid pAB1) and introduced it in RN4220 *S. aureus* cells. We then electroporated a linearized plasmid providing resistance to chloramphenicol (plasmid pC194) either in the presence or absence of anhydrotetracycline (aTc). Only cells that re-circularized the plasmid can form colonies on chloramphenicol plates (Supplementary Figure 4.a). We obtained 5 times more colonies when the NHEJ system was induced compared to the uninduced control (Supplementary Figure 4.b). Colonies were checked by sequencing the junction, which showed repair patterns typical of NHEJ (Supplementary Figure 4.c). We therefore concluded that the NHEJ system from *B. subtilis* was functional in *S. aureus.*

### Supplementary Note 2: Model for NHEJ system detection with MacsyFinder

```
<system inter_gene_max_space="5" min_mandatory_genes_required="1"
min_genes_required="1">
<gene name="ku" presence="mandatory" loner="1"/>
<gene name="ligD1" presence="accessory"/>
<gene name="ligD2" presence="accessory"/>
<gene name="ligD3" presence="accessory"/>
</system>
```

## Supplementary References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