

## Supplementary materials

### **CRISPR-dCas9 mediated cytosine deaminase base editing in *Bacillus subtilis***

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## **SUPPORTING INFORMATION**

Figure S1. Efficiency of plasmid curing.

Figure S2. Influence of different IPTG concentrations on the editing efficiency.

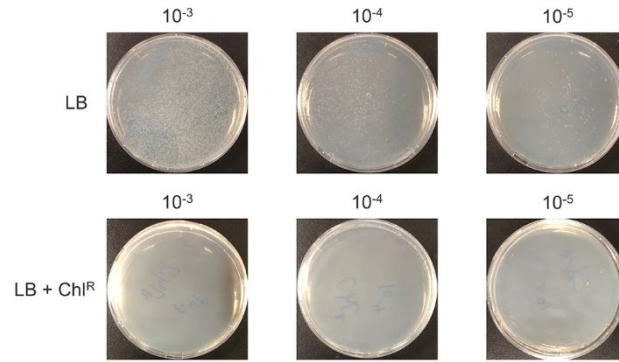
Figure S3. Multiplex genome editing efficiency for the inactivation of eight extracellular proteases.

Figure S4. Sequencing results for the verification of 8 protease genes deficient strain.

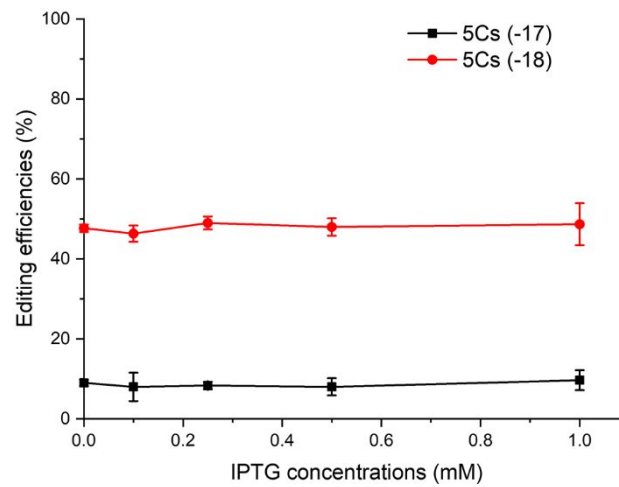
Table S1. Triple genome editing when subculture induction in LB liquid.

Table S2. Triple genome editing when subculture induction in LB plate.

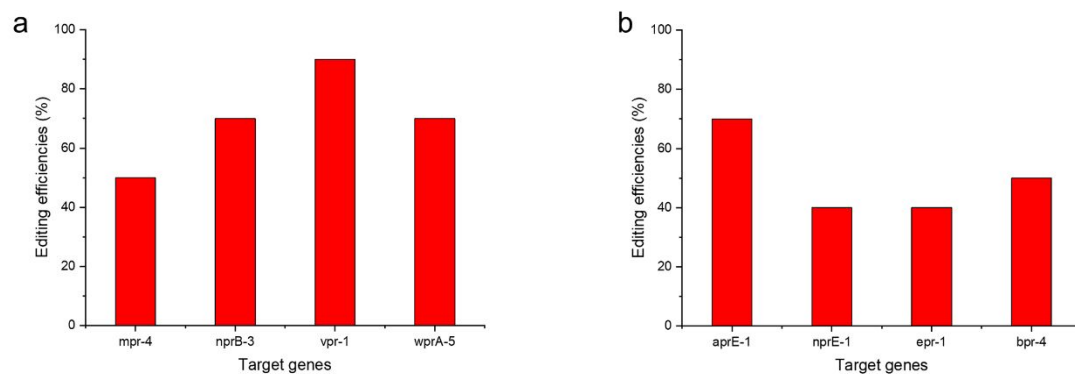
Table S3. Genome sequencing of the BS $\Delta$ 1-8Pro by NGS for off-target analysis.



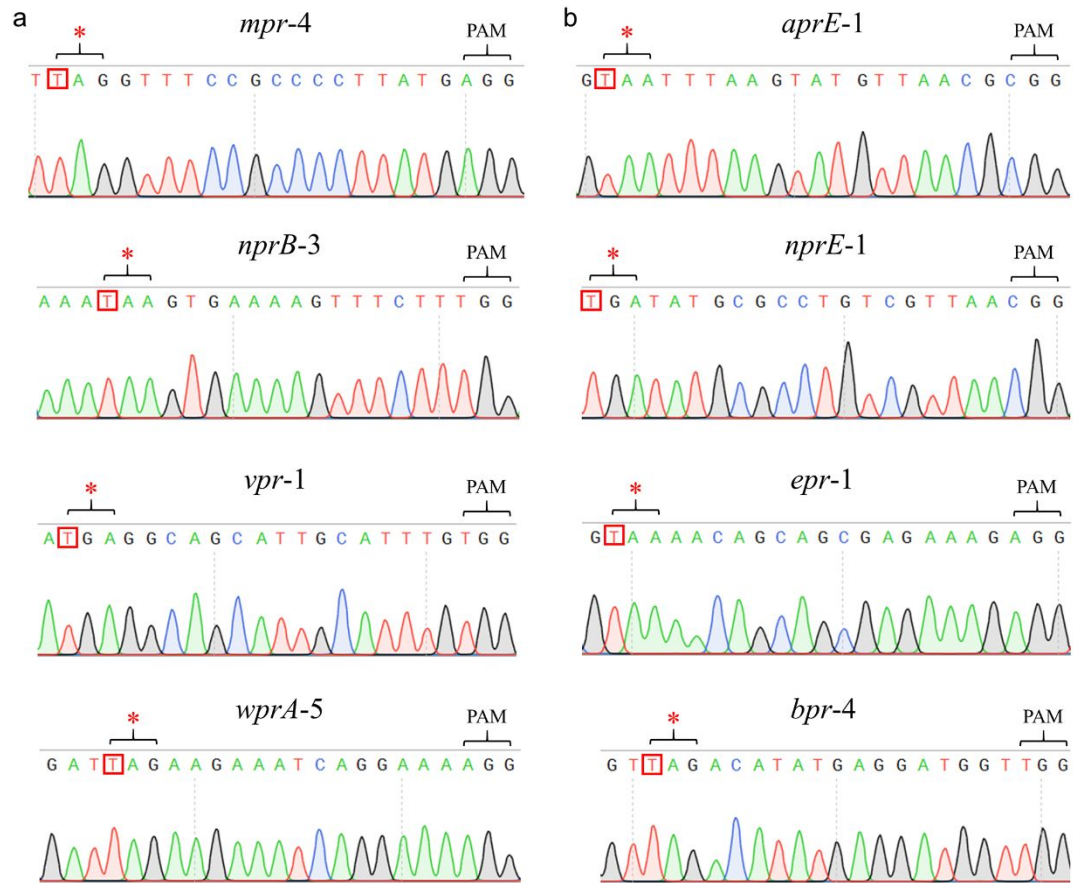
**Figure S1.** Efficiency of plasmid curing. All *B. subtilis* colonies were grown on antibiotic-free LB plates, but did not grow on LB chloramphenicol plates.



**Figure S2.** Influence of different IPTG concentrations on the editing efficiency. The final concentration of IPTG are 0, 0.1, 0.25, 0.5 and 1.0 mM.



**Figure S3.** Multiplex genome editing efficiency for the inactivation of eight extracellular proteases. (a) The first 4 proteases genes editing results. (b) The last 4 proteases genes editing results.



**Figure S4.** Sequencing results for the verification of 8 protease genes deficient strain. (a) The first 4 genes (*mpr*, *nprB*, *vpr* and *wprA*) deficient strain and (b) the last 4 genes (*aprE*, *nprE*, *epr* and *bpr*) deficient strain. Red square indicates edited base and red asterisk indicates early stop codon.

**Table S1.** Triple genome editing when subculture induction in LB liquid.

|    | 5Cs |     |     |     |     | <i>scoC</i> | <i>amyE</i> | Genes                         |
|----|-----|-----|-----|-----|-----|-------------|-------------|-------------------------------|
|    | -16 | -17 | -18 | -19 | -20 | -18         | -18         | Positions                     |
|    | 0   | 25% | 59% | 0   | 0   | 97%         | 100%        | Mixed culture                 |
| 1  | C   | C   | T   | C   | C   | C           | T           | Single colonies of subculture |
| 2  | C   | C   | C   | C   | C   | C           | T           |                               |
| 3  | C   | C   | C   | C   | C   | C           | T           |                               |
| 4  | C   | C   | C   | C   | C   | C           | T           |                               |
| 5  | C   | C   | C   | C   | C   | C           | T           |                               |
| 6  | C   | C   | C   | C   | C   | T           | T           |                               |
| 7  | C   | C   | C   | C   | C   | T           | C           |                               |
| 8  | C   | C   | C   | C   | C   | C           | T           |                               |
| 9  | C   | C   | C   | C   | C   | C           | C           |                               |
| 10 | C   | C   | C   | C   | C   | T           | C           |                               |

**Table S2.** Triple genome editing when subculture induction in LB plate.

|    | 5Cs |     |     |     |     | <i>scoC</i> | <i>amyE</i> | Genes                         |
|----|-----|-----|-----|-----|-----|-------------|-------------|-------------------------------|
|    | -16 | -17 | -18 | -19 | -20 | -18         | -18         | Positions                     |
|    | 0   | 25% | 67% | 0   | 0   | 85%         | 100%        | Mixed culture                 |
| 1  | C   | T   | T   | T   | C   | T           | T           | Single colonies of subculture |
| 2  | T   | T   | T   | C   | C   | T           | T           |                               |
| 3  | C   | T   | T   | C   | C   | T           | T           |                               |
| 4  | C   | T   | T   | T   | C   | T           | T           |                               |
| 5  | C   | T   | T   | C   | C   | T           | T           |                               |
| 6  | C   | T   | T   | C   | C   | T           | T           |                               |
| 7  | C   | T   | T   | C   | C   | T           | T           |                               |
| 8  | C   | T   | T   | C   | C   | T           | T           |                               |
| 9  | C   | T   | T   | C   | C   | T           | T           |                               |
| 10 | C   | T   | T   | C   | C   | T           | T           |                               |

**Table S3.** Genome sequencing of the BS△1-8Pro by NGS for off-target analysis.

| Gene Name                           | Gene Seq Change | Category           |
|-------------------------------------|-----------------|--------------------|
| <i>mpr</i>                          | c.181C>T        | Stop gained        |
| <i>yckA</i>                         | c.254G>A        | Missense variant   |
| <i>gabR</i>                         | c.1015G>A       | Missense variant   |
| <i>ydeQ</i>                         | c.440C>T        | Missense variant   |
| <i>groEL</i>                        | c.95C>T         | Missense variant   |
| <i>yerH</i>                         | c.1146C>T       | Synonymous variant |
| <i>mapB</i>                         | c.628G>A        | Missense variant   |
| <i>srtA</i>                         | c.188C>T        | Missense variant   |
| <i>aprE</i>                         | c.205C>T        | Stop gained        |
| <i>wprA</i>                         | c.541C>T        | Stop gained        |
| <i>nprB</i>                         | c.370C>T        | Stop gained        |
| <i>nprE</i>                         | c.280C>T        | Stop gained        |
| <i>bpr</i>                          | c.1324C>T       | Stop gained        |
| <i>pghZ</i>                         | c.479C>T        | Missense variant   |
| <i>accA</i>                         | c.421C>T        | Missense variant   |
| <i>murJ</i>                         | c.1515G>A       | Synonymous variant |
| <i>frlP</i>                         | c.1065C>T       | Synonymous variant |
| <i>minJ</i>                         | c.513C>T        | Synonymous variant |
| <i>nfi</i>                          | c.156G>A        | Synonymous variant |
| <i>narG</i>                         | c.2856C>T       | Synonymous variant |
| <i>spsK</i>                         | c.736C>T        | Missense variant   |
| <i>vpr</i>                          | c.1225C>T       | Stop gained        |
| <i>efeM</i>                         | c.294C>T        | Synonymous variant |
| <i>epr</i>                          | c.82C>T         | Stop gained        |
| Between <i>ymaB</i> and <i>cwlC</i> | n.1872798G>A    | Intragenic variant |
| Between <i>yuzF</i> and <i>yueE</i> | n.3264653C>T    | Intragenic variant |
| Between <i>degS</i> and <i>yvyE</i> | n.3646698C>T    | Intragenic variant |

The eight target genes are labeled green.