**Supplementary Data**

**Supplementary table 1.** List of primers

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| --- | --- | --- | --- |
| **Name** | **Sequence** | **Length** | **Purpose** |
| Fw\_BmpA\_8150 | GAGGCACTCAGTACTATGAAAAAACGCGTAATCG | 34 | Amplify BmpA gene, incorporated with labelling tags. |
| R1\_BmpA\_8150 | GATGGTGACCACTACCTTTTGAAGGAACAG | 30 | Amplify BmpA gene, incorporated with labelling tags. |
| Rv2\_ BmpA\_8150 | CAACAATGGTGATGGTGATGGTGACCACTACC | 32 | Amplify BmpA gene, incorporated with labelling tags. |
| Rv3\_ BmpA\_8150 | GCTTTTAGCAACAAGGACCGCAACAATGGTGAT | 33 | Amplify BmpA gene, incorporated with labelling tags. |
| R4\_BmpA\_8150 | AGCTTGAGCTCGCTTTTAGCAACAAGG | 27 | Amplify BmpA gene, incorporated with labelling tags. |
| Fw\_B26 | GCATAGGTCTCTGTACCATGAAAAAACGCGTAATCGCAG | 39 | Amplify N-terminal domains of truncated BmpA (B126, B66, B26) |
| Rv\_B26 | AATATGGTCTCATCATGTCATGTGAACGACATCCTGC | 37 | Amplify N-terminal domains of B26 |
| Rv\_B66 | AATATGGTCTCATCATCCCCCAGCTTTGAAGACCTTC | 37 | Amplify N-terminal domains of B66 |
| Rv\_B126 | AATATGGTCTCATCATAAAGTTAGATTTTGGATTATTTTTCGC | 43 | Amplify N-terminal domains of B126 |
| Fw\_U2 | GTAAAAACTGACCTTGCAGCAGCAATCGTCGCAGATGCTAATGGTGTAAATGACCG | 56 | mutation |
| Rv\_U2 | AAGGTCAGTTTTTACTTTACCTGAAGTTC | 29 | mutation |
| Fw\_U34 | GGATTCTCTTTAGCAGCAGCGACATCAGCAGCTGCGAAAAATAATC | 46 | mutation |
| Rv\_U34 | TAAAGAGAATCCAATACCAAACAAAAGTTTGTAAC | 35 | mutation |
| Fw\_D2 | CTTAAAGCAGCAATCGGCACCGATGCTAATGGTGTAAATGACCGTTC | 47 | mutation |
| Rv\_D2 | CGATTGCTGCTTTAAGGTCAGTTTTTACTTTAC | 33 | mutation |
| Fw\_D3 | CAACAAGGTTACAAAGGTGGTTTTGGTATTGGATTCTCTTTACAAGATGCG | 51 | mutation |
| Rv\_D3 | TTTGTAACCTTGTTGCTCCGCAGAGTTATAG | 31 | mutation |
| Fw\_II | CTCTTTAGCAGCAGCGATCATTGCAGCTGCGAAAAATAATCCAAAATC | 48 | mutation |
| Fw\_VV | CTCTTTAGCAGCAGCGGTTGTGGCAGCTGCGAAAAATAATCCAAAATC | 48 | mutation |
| Rv\_TS | CGCTGCTGCTAAAGAGAATCCAATACC | 27 | mutation |
| Fw\_BE7\_seq | ACAAGAATTAGTATTGCCTC | 20 | sequencing |
| Rv\_BE7\_seq | CACAGAATGCACACAAAGG | 19 | sequencing |
| Fw\_8150\_MSC | CTTAATTCTATCTTGAGAAAGTATTGG | 27 | sequencing |
| Rv\_8150\_MSC | GTAATTGCTTTATCAACTGCTGC | 23 | sequencing |
| Fw\_BmpA\_seq | CAGGTGTCTTCAGTGAAGC | 19 | sequencing |
| Rv\_BmpA\_seq | GGTCTTTAATGACAGAGTC | 19 | sequencing |

**Supplementary table 2.** List of L. lactis strains containing plasmids

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| **Strains** | **Original Strains** | **Plasmids** | **Protein of interest** |
| NpB | NZ9000 | pNZ8150:BmpA | Recombinant BmpA with 6-His & 4-Cys tags |
| NpBE1 | NZ9000 | pNZ8150:BE1 | *Fusion protein*: N-terminal 126 aa of BmpA with full-length E7 with 6-His & 4-Cys tags |
| NpBE2 | NZ9000 | pNZ8150:BE2 | *Fusion protein*: N-terminal 66 aa of BmpA with full-length E7 with 6-His & 4-Cys tags |
| NpBE3 | NZ9000 | pNZ8150:BE3 | *Fusion protein*: N-terminal 26 aa of BmpA with full-length E7 with 6-His & 4-Cys tags |
| NpBE1B | NZ9000 | pNZ8150:BE1B | BE1 fusion protein with modifications of Q111A and D112A in between H3 and H4 |
| NpBE1AB | NZ9000 | pNZ8150:BE1AB | BE1 fusion protein with combined modifications of K39A and T44A at H2 and Q111A and D112A in between H3 and H4 |
| NpBE1ABi | NZ9000 | pNZ8150:BE1ABi | BE1AB protein with more modifications at H4: T114I and T115I |
| NpBE1ABv | NZ9000 | pNZ8150:BE1ABv | BE1AB protein with more modifications at H4: T114V and T115V |
| NpBE1D | NZ9000 | pNZ8150: BE1D | BE1D fusion protein with modifications of L102G and L103G at H3 |
| NpBE1CD | NZ9000 | pNZ8150:BE1CD | BE1CD fusion protein with combined modifications of V43G at H2 and L102G and L103G at H3 |
| NpBE2A | NZ9000 | pNZ8150:BE2A | BE2 fusion protein with modifications of K39A and T44A at H2 |
| NpBE2C | NZ9000 | pNZ8150:BE2C | BE2 fusion protein with modifications of V43G at H2 |

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**a**

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**b**

Supplementary Figure 1. Overproduction of fusion proteins three hours post-induction. (a) Overproduction of hybrid fusion proteins on the surface, displayed by the overlay histogram of unstained population (grey) and stained population (green) in flow cytometry. (b) Overproduction of intracellular fusion proteins, displayed by overlay as scaled viewer image of two microscopic channels (brightfield and red fluorescence). The order from left to right is NpBE1, NpBE2, NpBE3.

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Supplementary Figure 2. Surface expression of cells carrying modified fusion proteins (alanine). Each individual histogram displays the peaks of unstained sample (grey peak) and stained sample (green peak). In the overlay histogram, all unstained and stained samples were combined, in which all unstained samples were displayed by grey colour and stained samples were displayed by different colours.

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| **A picture containing text, nature  Description automatically generated** | **A picture containing nature, rain, different  Description automatically generated** | A picture containing text, nature, people  Description automatically generated | A picture containing text, nature  Description automatically generated | Graphical user interface, application  Description automatically generated |
| **A picture containing dark, night sky  Description automatically generated** | **A picture containing star, dark, outdoor object, night  Description automatically generated** | **Red lights in the dark  Description automatically generated with medium confidence** | Red lights in the dark  Description automatically generated | Red lights in the dark  Description automatically generated with low confidence |
| BE1 3H | BE1B 3H | BE1AB 3H | BE1D 3H | BE1CD 3H |
| A picture containing nature, game, rain, controller  Description automatically generated | A picture containing text, nature, crater  Description automatically generated | A picture containing nature, rain, game, hand  Description automatically generated | A picture containing nature, game, hand  Description automatically generated | A picture containing nature, rain, different, game  Description automatically generated |
| **A black rectangle with a black background  Description automatically generated with low confidence** | **A picture containing dark, night, night sky  Description automatically generated** | **A picture containing dark, night, night sky  Description automatically generated** | A black rectangle with a black background  Description automatically generated with low confidence  BE1D 24H | A picture containing dark, night sky  Description automatically generated |
| BE1 24H | BE1B 24H | BE1AB 24H |  | BE1CD 24H |

**b**

**a**

|  |  |  |  |  |  |  |
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| **a** (cont.) | A picture containing nature  Description automatically generated | A picture containing game  Description automatically generated | **c** | A picture containing text, nature  Description automatically generated | Graphical user interface, application  Description automatically generated | A picture containing text, people  Description automatically generated |
|  | Red lights in the dark  Description automatically generated with low confidence | Red lights in the dark  Description automatically generated with low confidence |  | A picture containing dark, night sky  Description automatically generated | Red lights in the dark  Description automatically generated with medium confidence | A picture containing dark, star, night, night sky  Description automatically generated |
|  | BE1ABi 3H | BE1ABv 3H |  | BE2 3H | BE2A 3H | BE2C 3H |
|  | A picture containing nature, rain, game, different  Description automatically generated | A picture containing nature, game, different  Description automatically generated | **d** | A picture containing game, controller, remote, playing  Description automatically generated | A picture containing nature, different, crater  Description automatically generated | A picture containing nature, different, game  Description automatically generated |
| **b** (cont.) | A picture containing dark, night, star, outdoor object  Description automatically generated | **A picture containing star, dark, outdoor object, night  Description automatically generated**  BE1ABv 24H |  | A picture containing star, dark, outdoor object, night  Description automatically generated | **A picture containing night sky  Description automatically generated**  BE2A 24H | Shape  Description automatically generated with medium confidence  BE2C 24H  24H |
|  | BE1ABi 24H |  |  | BE2 24H |  |  |

Supplementary Figure 3. Intracellular fluorescence expression of all cell strains producing fusion proteins with modified hydrophobic patches (a) NpBE1 variations at 3 hours, (b) NpBE1 variations at 24 hours, (c) NpBE2 variations at 3 hours, (d) NpBE2 variations at 24 hours.