

**Supplementary Table S2: Peptidoglycan hydrolases screened for high activity in murine serum.** All constructs are part of the strain collection of the Laboratory of Food Microbiology (ETH, Zurich).

|                              | ID     | Name                                   | Plasmid <sup>1</sup> | <i>E. coli</i> expression strain |
|------------------------------|--------|--|----------------------|----------------------------------|
| Gly-Gly Endopeptidase        | PGH 1  | H_(M23LST)2_LST                        | pQE30                | SURE                             |
|                              | PGH 2  | M23LST(L)_SH3b2638                     | pET9a                | BL21-Gold(DE3)                   |
|                              | PGH 3  | H_(LST)2                               | pQE30                | SURE                             |
|                              | PGH 4  | H_LST_M23LST                           | pQE30                | SURE                             |
|                              | PGH 5  | H_TEV_(M23LST)2_SH3bLST                | pQE30                | SURE                             |
|                              | PGH 6  | (M23LST)2_SH3b2638                     | pET302 <sup>1</sup>  | BL21-Gold(DE3)                   |
|                              | PGH 7  | (M23LST)2_SH3bAle1                     | pET302               | BL21-Gold(DE3)                   |
| Gly-D-Ala Endopeptidase      | PGH 8  | CHAPGH15_SH3bAle1                      | pET302               | BL21-Gold(DE3)                   |
|                              | PGH 9  | CHAPT <sub>w</sub> (L)_SH3b2638        | pET302               | BL21-Gold(DE3)                   |
|                              | PGH 10 | H_CHAP-1_CBD-1                         | pET302               | BL21-Gold(DE3)                   |
|                              | PGH 11 | H_TEV_CHAPK_SH3bLST                    | pQE30                | XL1-Blue MRF <sup>1</sup>        |
|                              | PGH 12 | CHAPK_SH3bLST_H                        | pET21a               | BL21-Gold(DE3)                   |
|                              | PGH 13 | CHAPGH15_SH3b2638                      | pET302               | BL21-Gold(DE3)                   |
|                              | PGH 14 | CHAPSEP1(L)_SH3b2638                   | pET302               | BL21-Gold(DE3)                   |
|                              | PGH 15 | (CHAPGH15)2_SH3bAle1                   | pET302               | BL21-Gold(DE3)                   |
|                              | PGH 16 | H_TEV_(CHAPK)2_SH3bLST                 | pQE30                | SURE                             |
|                              | PGH 17 | (CHAPGH15(L))2_SH3b2638                | pET302               | BL21-Gold(DE3)                   |
| Double EAD mixed specificity | PGH 18 | CHAPT <sub>w</sub> _M23LST(L)_SH3b2638 | pET302               | BL21-Gold(DE3)                   |
|                              | PGH 19 | CHAPK_AmiK_SH3bLST_H                   | pET21a               | BL21-Gold(DE3)                   |
|                              | PGH 20 | LysK_H                                 | pET21a               | BL21-Gold(DE3)                   |
|                              | PGH 21 | M23LST(L)_Ami2638_SH3b2638             | pET200               | BL21-Gold(DE3)                   |
|                              | PGH 22 | M23LST(L)_Ami2638(L)_SH3b2638          | pET200               | BL21-Gold(DE3)                   |
| Triple EAD mixed specificity | PGH 23 | LST_CHAPK_AmiK_H                       | pET21a               | BL21-Gold(DE3)                   |
|                              | PGH 24 | LST_CHAPH5_AmiH5(L)_H                  | pET21a               | BL21-Gold(DE3)                   |
|                              | PGH 25 | LysK_LST_H                             | pET21a               | BL21-Gold(DE3)                   |
|                              | PGH 26 | Pep $\lambda$ SA2_CHAPK_LST_H          | pET21a               | BL21-Gold(DE3)                   |
|                              | PGH 27 | CHAP11_Ami11_LST_H                     | pET21a               | BL21-Gold(DE3)                   |
|                              | PGH 28 | CHAPH5_AmiH5_LST_H                     | pET21a               | BL21-Gold(DE3)                   |

<sup>1</sup> Resistance encoded on plasmid: ampicillin on pQE30, pET302 and pET21a; kanamycin on pET9a and pET21a.

Abbreviations: EAD, enzymatically active domain; H, 6x histidine tag; PGH, peptidoglycan hydrolase.