Supplementary Material and Methods

Construction of *L. rhamnosus* ATCC 53103 Δ*luxS*

To delete *luxS*, we applied the vancomycin-based counterselection system (pVPL3002) as described by Zhang et al. [1]. First, we cloned the upstream and downstream flanks of *luxS* in pVPL3002 by Ligase Cycling Reaction (LCR)[2]. The plasmid backbone of pVPL3002 was amplified with oVPL187-188, and oligonucleotide pairs oVPL3228-3229 and oVPL3230-3231 were used to amplify the up- and downstream flanks of *luxS*, respectively. The three amplicons were fused using bridging oligonucleotides oVPL3232, 3233, and 3234. The resulting plasmid construct was named pVPL31157. We transformed 3 μg pVPL31157 in *L. rhamnosus* ATCC 53103 as described in Zhang et al. We used oligonucleotide pairs oVPL49-3235-3236 and oVPL97-3235-3236 to identify upstream and downstream integration of pVPL31157, respectively. Upon confirmation of single-crossover homologous recombination (SCO), cells were cultured in MRS for 20 generations in the absence of antibiotics and plated on MRS agar containing 1,000 μg/mL vancomycin. This selects for cells that have undergone a second homologous recombination event. By using PCR (oligonucleotides oVPL3235-3236) we screened for deletion of *luxS*. Efficiency of the deletion was subsequently verified by Sanger sequencing. The resultant *luxS* mutant strain was named VPL4310. The strains, plasmids, and oligonucleotides used for *luxS* mutant construction are detailed in Table 1 and 2.
## Supplementary Table S1. Bacterial strains and plasmids used in this study

| Genus and Species       | Strain$^a$   | Description$^b$                      | Source$^c$ |
|-------------------------|--------------|--------------------------------------|------------|
| *Escherichia coli*      | EC1000       | In trans RepA provider, Kan$^{\text{R}}$ (cloning host) | [3]        |
| *Escherichia coli*      | VPL3002      | EC1000 harboring pVPL3002, Em$^{\text{R}}$ | [1]        |
| *Escherichia coli*      | VPL31157     | EC1000 harboring pVPL31157, Em$^{\text{R}}$ | This study |
| *Lactobacillus rhamnosus* | ATCC 53103 | Wild-type                           | ATCC      |
| *Lactobacillus rhamnosus* | VPL4310 | ATCC 53103 ΔluxS                      | This study |

| Plasmids | Genotype | Description | Source |
|----------|----------|-------------|--------|
| pVPL3002 | pORI19::ddlA F258Y$_{r_{euteri}}$, Em$^{\text{R}}$ | Suicide shuttle vector with vancomycin counter-selection marker | [1] |
| pVPL31157 | pVPL3002::luxS deletion cassette, Em$^{\text{R}}$ | Deletion cassette targets luxS in ATCC 53103 | This study |

$^a$: VPLxxxx: Van Pijkeren Laboratory strain collection identification number; $^b$: Kan$^\text{R}$: kanamycin resistance; Em$^\text{R}$: erythromycin resistance; pVPLxxxx: Van Pijkeren Lab plasmid collection identification number; $^c$: ATCC: American Type Culture Collection
## Supplementary Table S2. Oligonucleotides used in this study

| Oligonucleotides<sup>#</sup> | Sequence (5’-3’) | Description<sup>‡</sup> |
|-------------------------------|-----------------|--------------------------|
| oVPL49                        | acaatttcacaggaacagc | Oligo paired with oVPL97 used for screening pVPL3002 constructs |
| oVPL97                        | cccccattaagtgccagtgc | Oligo paired with oVPL49 used for screening pVPL3002 constructs |
| oVPL187                       | taccgagctgtaatcaaggg | Rev, internal oligo for pVPL3002 backbone amplification |
| oVPL188                       | atctctagagtagctgagc | Fwd, internal oligo for pVPL3002 backbone amplification |
| oVPL3228                      | ttagctgatgtagtgcaaggc | Fwd, paired with oVPL3229 used for luxS gene deletion cassette (u/s) |
| oVPL3229                      | taagcgccttaactgcaggtg | Rev, paired with oVPL3228 used for luxS gene deletion cassette (u/s) |
| oVPL3230                      | attaccggcagggtgtctataatc | Fwd, paired with oVPL3231 used for luxS gene deletion cassette (d/s) |
| oVPL3231                      | gttcgttttagctgctgtc | Rev, paired with oVPL3230 used for luxS gene deletion cassette (d/s) |
| oVPL3232                      | aaacgacggccagtggaacctagctgattagtctgatgtagtgcaaggcgcaagcataacctgggca | Bridging oligonucleotides used for LCR |
| oVPL3233                      | gtagataccactgcagtaagggccttaattaccggcagtgtcatactgacga | Bridging oligonucleotides used for LCR |
| oVPL3234                      | cgattggaaacagcagggataacaagaaacacactetccttagtagctgacagcagcagtattaatcggcaagc | Bridging oligonucleotides used for LCR |
| oVPL3235                      | ggctttactggcacttgcagcaggcatgtaatcggcagcagcagtattaatcggcaagc | Bridging oligonucleotides used for LCR |
| oVPL3236                      | gttgagcagctggctgtaaatcggcagcagcagtattaatcggcaagc | Bridging oligonucleotides used for LCR |

<sup>#</sup>: oVPLxxxx: Van Pijkeren Laboratory oligonucleotide identification number; <sup>‡</sup>: fwd: forward; rev: reverse; u/s: upstream; d/s: downstream; LCR: ligation cycling reaction
## Supplementary data

### Supplementary Table S3. Primer sequences for RT-qPCR

| Primers  | 5'-3'       | Sequence                                    |
|----------|-------------|---------------------------------------------|
| **β-actin** | Forward  | GTGCCCATCTATGAGGTACGCT                      |
|          | Reverse    | GTCACGGACAATTTCCTCTTTCGGG                 |
| **TLR1** | Forward  | TAAACCTTCGGCACAACCCGA                      |
|          | Reverse    | AGATCCAGCAGCGGTATGAA                      |
| **TLR2** | Forward  | AAACCTGCTGTCGTATGACGCTT                   |
|          | Reverse    | ACACAGGGAACACAGAGCT                      |
| **TLR3** | Forward  | TTTCTGGCTTCGGGACCT                        |
|          | Reverse    | ACTTTGTGATGCCCATGCT                      |
| **TLR4b** | Forward  | TACCACTTGGGTGCTCTGAG                      |
|          | Reverse    | AATGTTGATCCGTACGCT                        |
| **TLR5b** | Forward  | AGAGACGGCGCGGTATGAG                      |
|          | Reverse    | GAAGCTGGCTGGATTTTCTGT                     |
| **TNF-α** | Forward  | GTGCAATCCGCTCAATCTGCACG                  |
|          | Reverse    | AATGGAAGGCACGGCGAGG                      |
| **IL-1β** | Forward  | GGCACTGCTGTCGCTGACG                      |
|          | Reverse    | GGGGCAACACAGGCGAGG                      |
| **IL-6** | Forward  | ATGAGCGGATCTGAGGG                        |
|          | Reverse    | GCAGCGGTCTGAAGGT                           |
| **NF-κB** | Forward  | TTTCCGAGGAGAGATGGAGAG                      |
|          | Reverse    | CTGTTCAAGTGAGCGAG                        |
| **IκBα** | Forward  | TTTCGGAGGAGAGATGGAGAG                      |
|          | Reverse    | CTGTTCAAGTGAGCGAG                        |
| **MyD88** | Forward  | GAGGATGGTGTTGATCCT                       |
|          | Reverse    | CGACAGGAGATTAGCAGCTG italic                 |
| **STAT** | Forward  | ATCGACCTTGGAGACGACT                       |
|          | Reverse    | CCCATCGGCTTGGGAGCTG italic                |
| **JNK**  | Forward  | GGAATAGAATGATGTTGICTGGATGATG            |
|          | Reverse    | TGGTTCTGGGAGGCTCGTCTGAG                  |
| **p38**  | Forward  | CCTGAAGATCATGCTCAACCTG                   |
|          | Reverse    | GCTAGGACATCTGCTTTATTAGAGA                |

## References

1. Zhang, S., et al., *D-Ala-D-Ala ligase as a broad host-range counterselection marker in vancomycin-resistant lactic acid bacteria*. Journal of bacteriology, 2018: p. JB. 00607-17.

2. Kok, S.d., et al., *Rapid and reliable DNA assembly via ligase cycling reaction*. ACS synthetic biology, 2014. 3(2): p. 97-106.

3. Leenhouts, K., et al., *Construction of a food-grade multiple-copy integration system for Lactococcus lactis*. Applied Microbiology and Biotechnology, 1998. 49(4): p. 417-423.
**Supplementary Figure S1.** Expression of TLRs of germ-free (GF), conventionally raised (CR), and LGG-colonized zebrafish larvae, n=6. GF, WT and ΔluxS, zebrafish larvae in germ-free were exposed to none or to wild-type or ΔluxS LGG at a concentration of 10^8 cfu/mL for 24 h on 5 dpf, respectively. TLR, toll-like receptor, p < 0.05 (*), p < 0.01 (**) and p < 0.001 (***)

**Supplementary Figure S2.** The deletion base of the luxS gene.

| WT.txt | ACTTAAAGCCCGCTTAACTGCCGTATTACCCACCGAARATTGCCGGGATGGAATTTCAATTGAATTACGGCT |
| luxS.txt | ACTTAAAGCCCGCTTAACTGCCGTATTACCCACCGAARATTGCCGGGATGGAATTTCAATTGAATTACGGCT |
| Consensus | AGTAAAGCGGGCAACGAACCGTCCGATATGCGCACACTGGGCATATATATGTCGGC |

| WT.txt | TGTTTCAACGCAACACCGGCGATGATCATCAGCGGCGGTGCAACAATTCGACTGCTTGTGGCGGTATTTTA |
| luxS.txt | TGTTTCAACGCAACACCGGCGATGATCATCAGCGGCGGTGCAACAATTCGACTGCTTGTGGCGGTATTTTA |
| Consensus | ACCGTTCAACGCAACACCGGCGATGATCATCAGCGGCGGTGCAACAATTCGACTGCTTGTGGCGGTATTTTA |

| WT.txt | GCAGTGGATGGCTGATTGGCGGTGCTTGGCGGTATGATCAGCAGCTGCTGCGATGGAATTTCAATTGAATTACGGCT |
| luxS.txt | GCAGTGGATGGCTGATTGGCGGTGCTTGGCGGTATGATCAGCAGCTGCTGCGATGGAATTTCAATTGAATTACGGCT |
| Consensus | AGTAAAGCGGGCAACGAACCGTCCGATATGCGCACACTGGGCATATATATGTCGGC |

| WT.txt | AGCTGGGAAGGGCAACCGTCCGATATGCGCACACTGGGCATATATATGTCGGC |
| luxS.txt | AGCTGGGAAGGGCAACCGTCCGATATGCGCACACTGGGCATATATATGTCGGC |
| Consensus | AGTAAAGCGGGCAACGAACCGTCCGATATGCGCACACTGGGCATATATATGTCGGC |

| WT.txt | ATTTCCGAGACGGCACAGTTTGACGCAATCAGGACAGATCAACAAAAATAGCACACAGCAATTT |
| luxS.txt | ATTTCCGAGACGGCACAGTTTGACGCAATCAGGACAGATCAACAAAAATAGCACACAGCAATTT |
| Consensus | ATTTCCGAGACGGCACAGTTTGACGCAATCAGGACAGATCAACAAAAATAGCACACAGCAATTT |