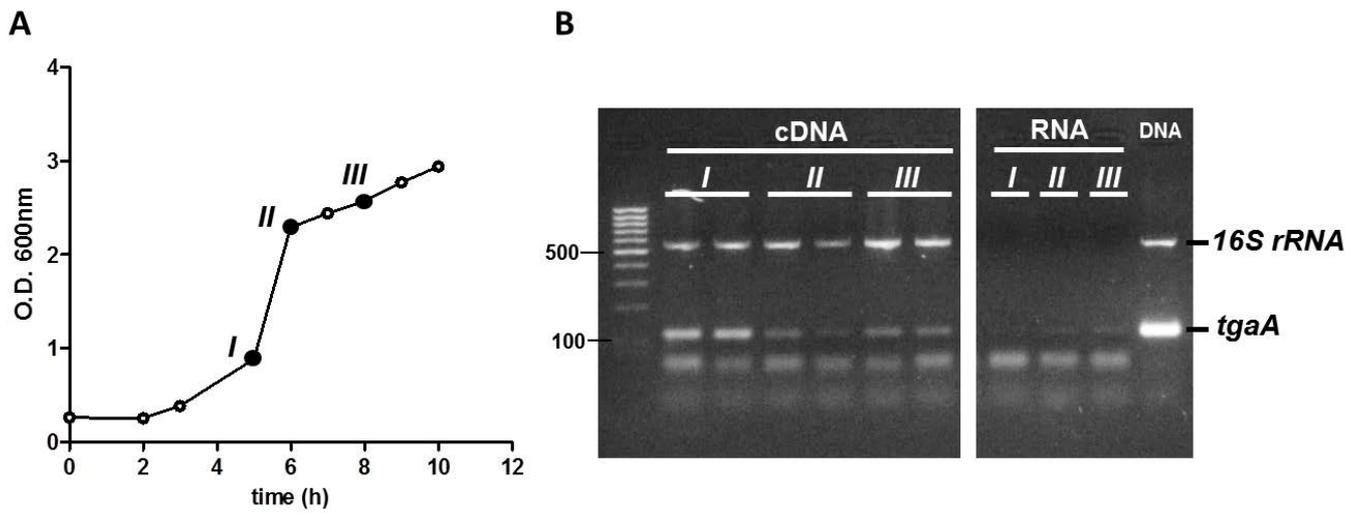
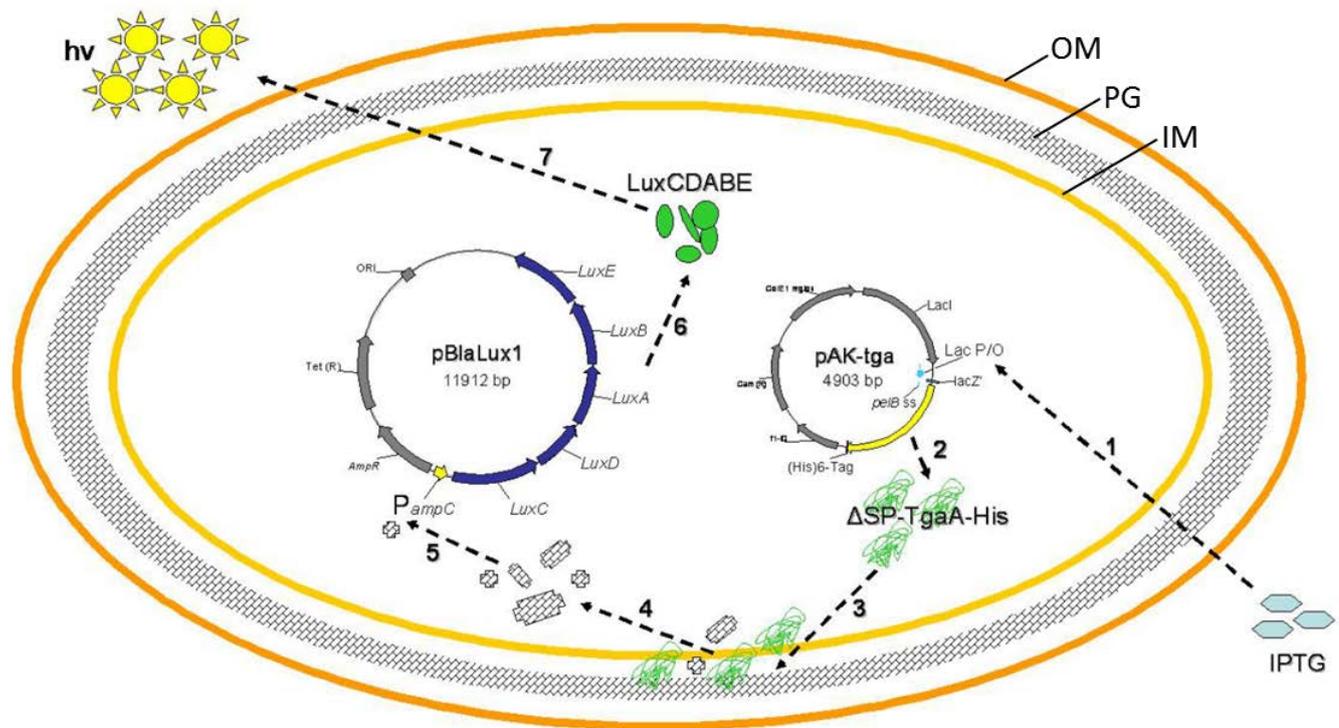


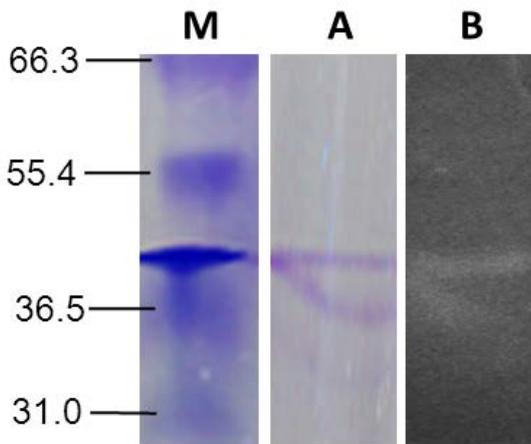
**Supplementary Figure S1.** Expression analysis of *tgaA* gene. Panel A, growth curve of *Bifidobacterium bifidum* MIMBb75 in cMRS broth (2% inoculum, anaerobic incubation, 37°C). Panel B, semiquantitative RT-PCR of *tgaA* in cells collected at the mid-exponential (I), late-exponential (II) and stationary phase (III). 16S rRNA transcripts were used as internal control. PCR fragments of *tgaA* and 16S rRNA amplified from the same cDNA sample were loaded in the same well of a 3% agarose gel and separated by electrophoresis. Duplicates of cDNA amplifications are shown.



**Supplementary Figure S2.** Functioning of the double expression reporter system for the determination of the peptidoglycan lytic activity of TgaA. The vector pAK-tga expressing SP<sub>PeIB</sub>-TgaA was introduced by electrotransformation in an *E. coli* SNO301 mutant that contains pBlaLux1, a bioluminescence reporter vector activated by peptidoglycan fragments (S.J. Valtonen et al., J. Biomol. Screen. 7:127-134, 2002). The addition of IPTG to the double reporter system [1] induces the expression from pAK-tga of recombinant TgaA [2], which is exported to periplasm [3] due to the presence of PelB signal peptide. Once in the periplasm, SPPeIB-TgaA liberates cell-wall breakdown products that accumulate in the cell [4] activating the PampC regulatory region [5]. The activation of the PampC promoter determines the expression of lux operon [6] and the production of bioluminescence [7]. OM, outer membrane; PG, peptidoglycan; IM, inner membrane.

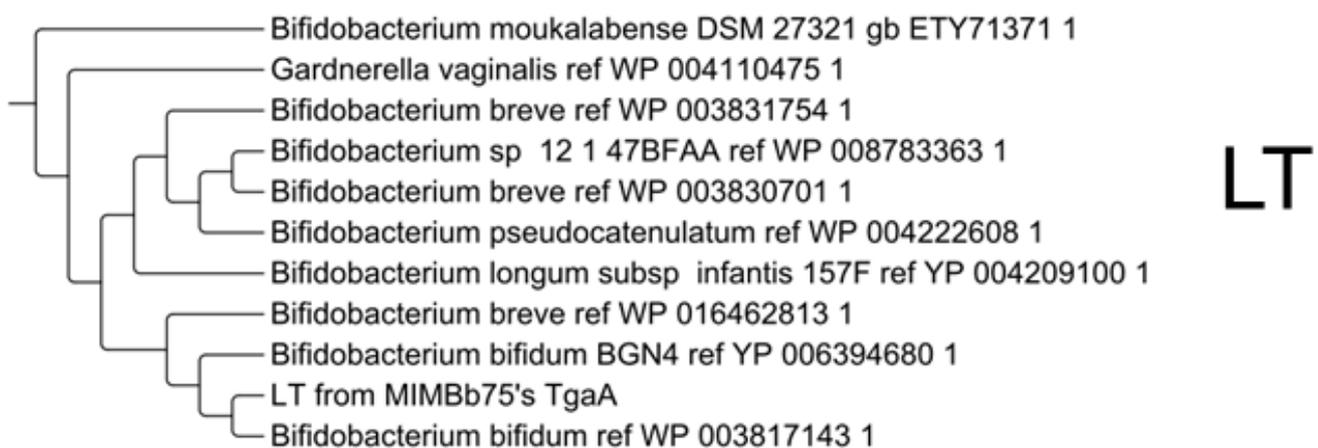


**Supplementary Figure S3.** SDS-PAGE (panel “A”) and renaturing SDS-PAGE containing 0.2% autoclaved *M. luteus* cells (panel “B”) of the purified six-His-tagged SP<sub>PelB</sub>-TgaA recombinant protein. M, molecular marker (molecular masses in kDa are indicated on the left).



**Supplementary Figure S4.** Distance trees (Fast Minimum Evolution method with a maximum sequence difference of 0.85 and Grishin distance) produced using BLAST pairwise alignments between LT (panel a) or CHAP (panel b) domain aminoacidic sequences used as queries, and the database sequences searched.

a)



b)

CHAP

