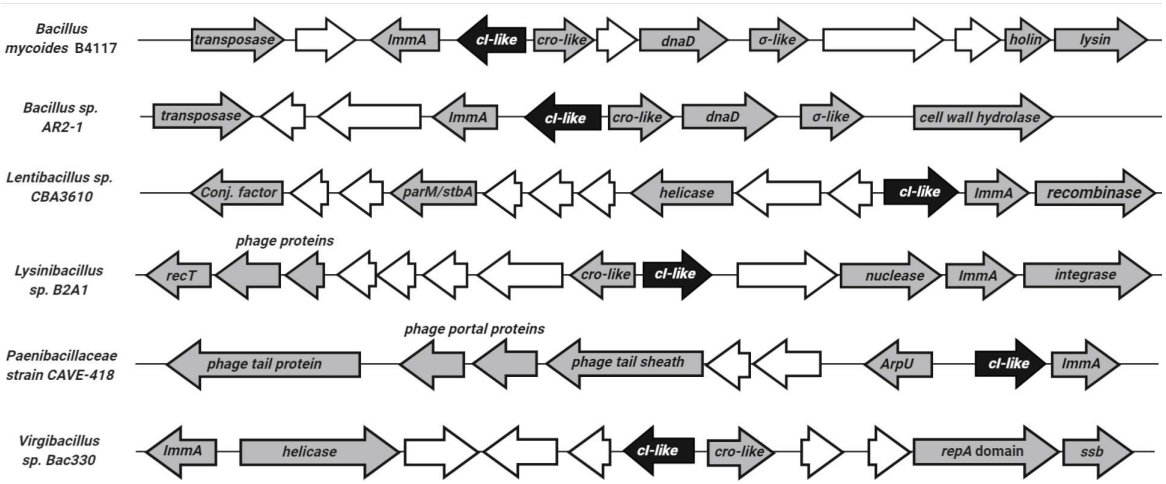
Supplementary Figure S1

A.



B.

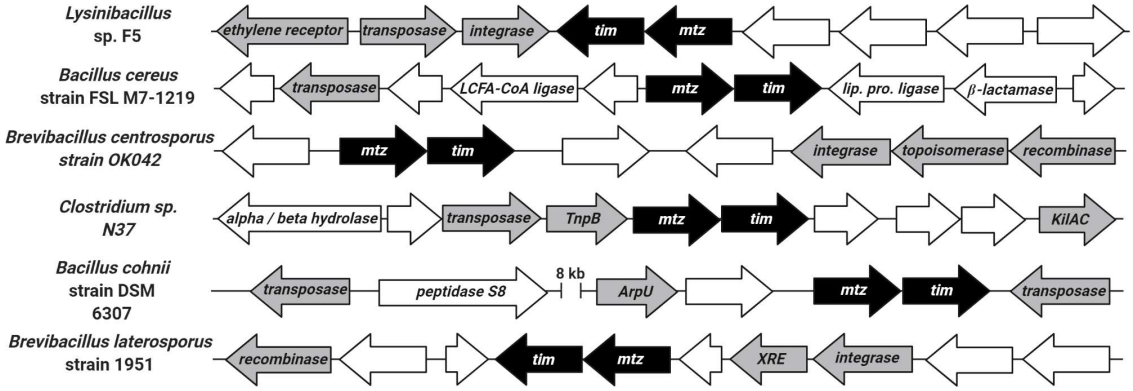


Figure S1. (A) **Bioinformatic analysis of *cI*-like homologues in Gram-positive bacteria (Firmicutes).** Schematic representation of the *cI-like* homologues (~ 60% similarity, not including the HTH domain, belonging to the HipB superfamily) and proximal genes in Firmicutes bacteria related to *Lm*. The *cI-like* homologues are found proximal to mobile genetic element-associated genes and *immA* antirepressors. Black arrows represent the *cI-like* homologues, gray arrows represent mobile genetic element genes. (B) In Firmicutes bacteria related to *Listeria,* gene pairs highly similar to the metzincin-TIMP-like pair are found proximal to mobile genetic element genes. Black arrows represent the *cro-cI*-like/metzincin-TIMP-like genes, gray arrows represent mobile genetic element genes.

Supplementary Figure S2

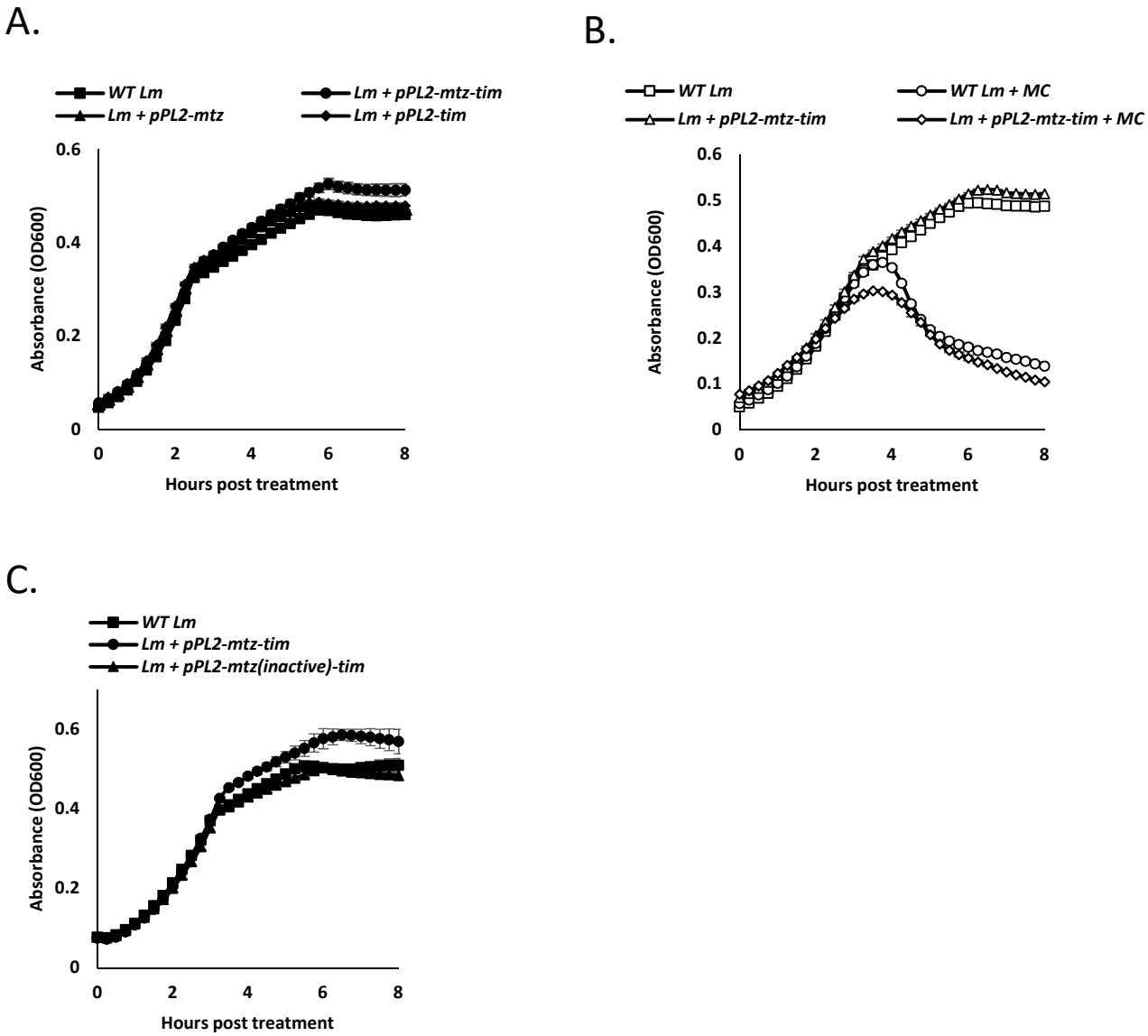


Figure S2. Ectopic expression of Mtz-Tim does not affect *Lm* growth in BHI at 37°C. (A) Growth analysis in BHI media of WT *Lm*, and WT *Lm* harboring pPL2 integrative plasmid expressing *mtz, tim* or both from an inducible p_{tetR} promoter (pPL2-*mtz,* pPL2-*tim*, and pPL2-*mtz-tim* respectively) at 37°C. (B) Growth analysis in BHI media of WT *Lm*, and WT *Lm* harboring pPL2 integrative plasmid expressing *mtz* and *tim* from an inducible p_{tetR} promoter (pPL2-*mtz-tim*) with and without MC treatment at 30°C. (C) Growth analysis in BHI media of WT *Lm*, and WT *Lm* harboring pPL2 integrative plasmid expressing *mtz-tim* or *mtz* mutated at position E136A with *tim* from an inducible p_{tetR} promoter (pPL2-*mtz-tim* and pPL2-*mtz(inactive)-tim*, respectively) at 37°C. Data in A-C represent three independent experiments and error bars represent the standard deviation of triplicates.

Supplementary Figure S3

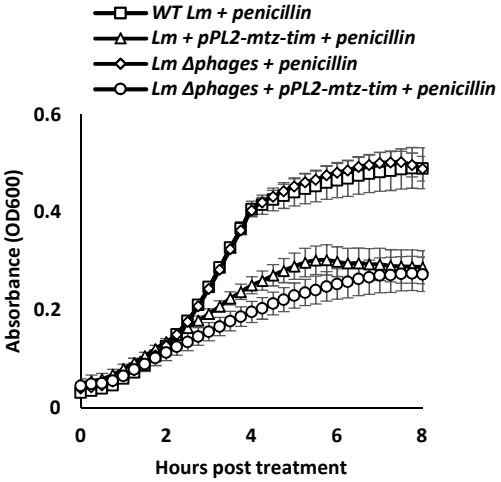
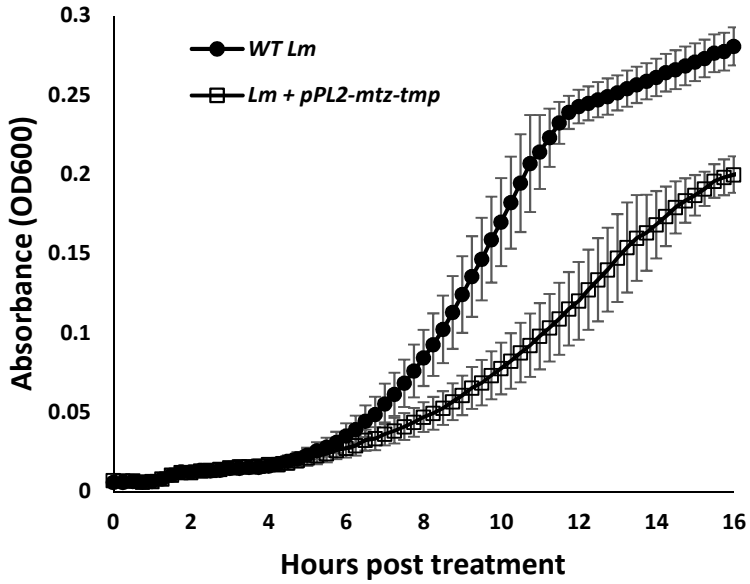


Figure S3. The sensitivity of Mtz-Tim expressing bacteria to penicillin is independent of the phage elements. Growth analysis in BHI media of *Lm* strain deleted of the phage elements (*Δphages*) and WT *Lm* harboring pPL2 plasmid expressing *mtz-tim* from an inducible p_{tetR} promoter (pPL2-*mtz-tim*) at 37°C, with and without addition of penicillin. Data represent three independent experiments and error bars represent the standard deviation of triplicates.

Supplementary Figure S4

A.



B.

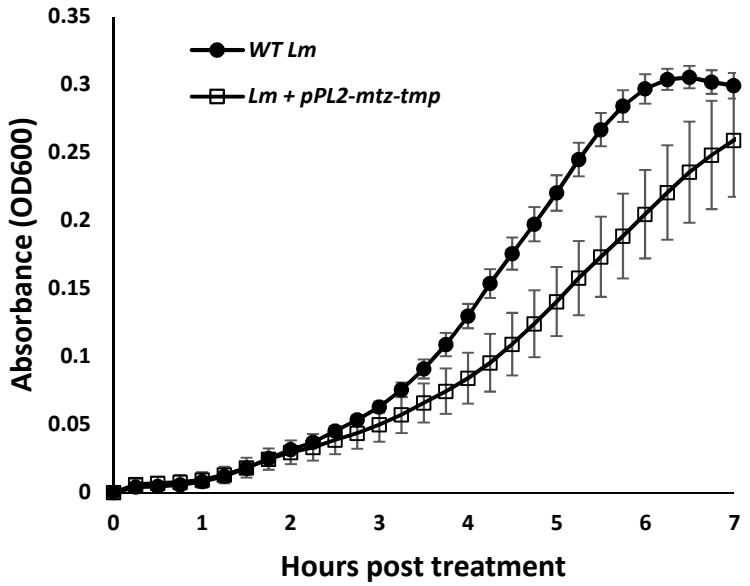


Figure S4. Ectopic expression of Mtz-Tim sensitizes *Lm* to cell-wall acting antibiotics. Growth analysis in BHI media of WT *Lm* and WT *Lm* harboring pPL2 plasmid expressing *mtz-tim* from an inducible p_{tetR} promoter (pPL2-*mtz-tim*) at 37°C, with and without addition of 250 µg/ml of bacitracin (A) and 12 µg/ml D-cycloserine (B). Data represent an average of three independent experiments and error bars represent the standard deviation.

Supplementary Figure S5

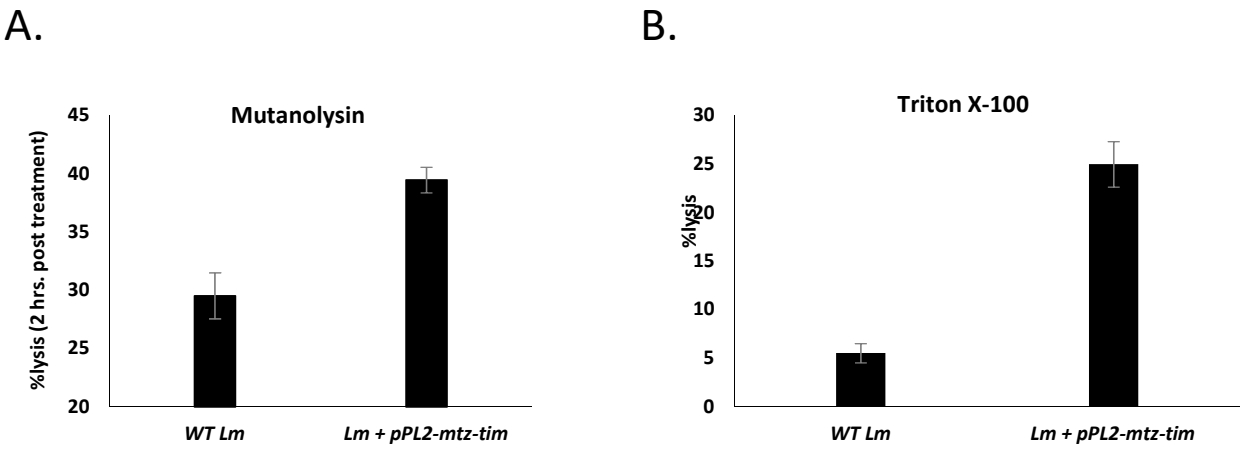


Figure S5. Ectopic expression of Mtz-Tim sensitizes *Lm* to lysis caused by mutanolysin or Triton X-100. Growth analysis in BHI media at 37°C of WT *Lm*, and WT *Lm* harboring pPL2 plasmid expressing *mtz-tim* from an inducible p_{tetR} promoter (pPL2-*mtz-tim*) with and without addition of mutanolysin (A) or Triton X-100 (B). Data is presented as percentage of lysis at 2 hours after treatment. The data represent three independent experiments, error bars represent the standard deviation of triplicates.