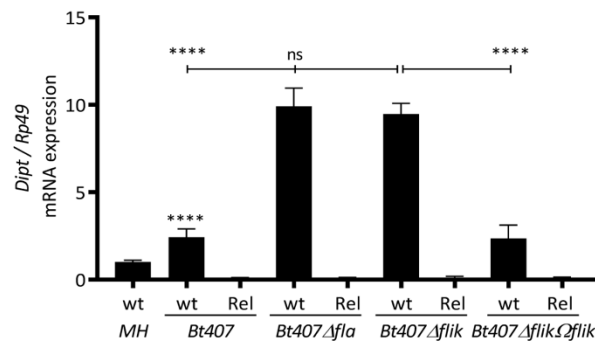
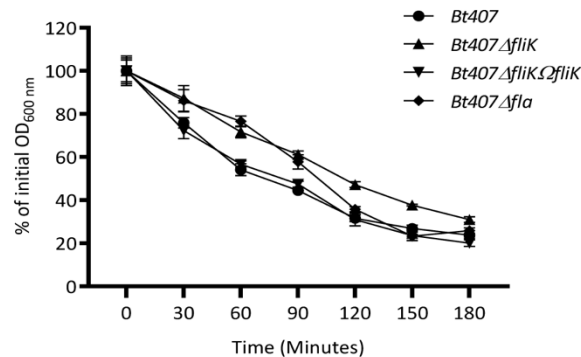


Supplementary Material

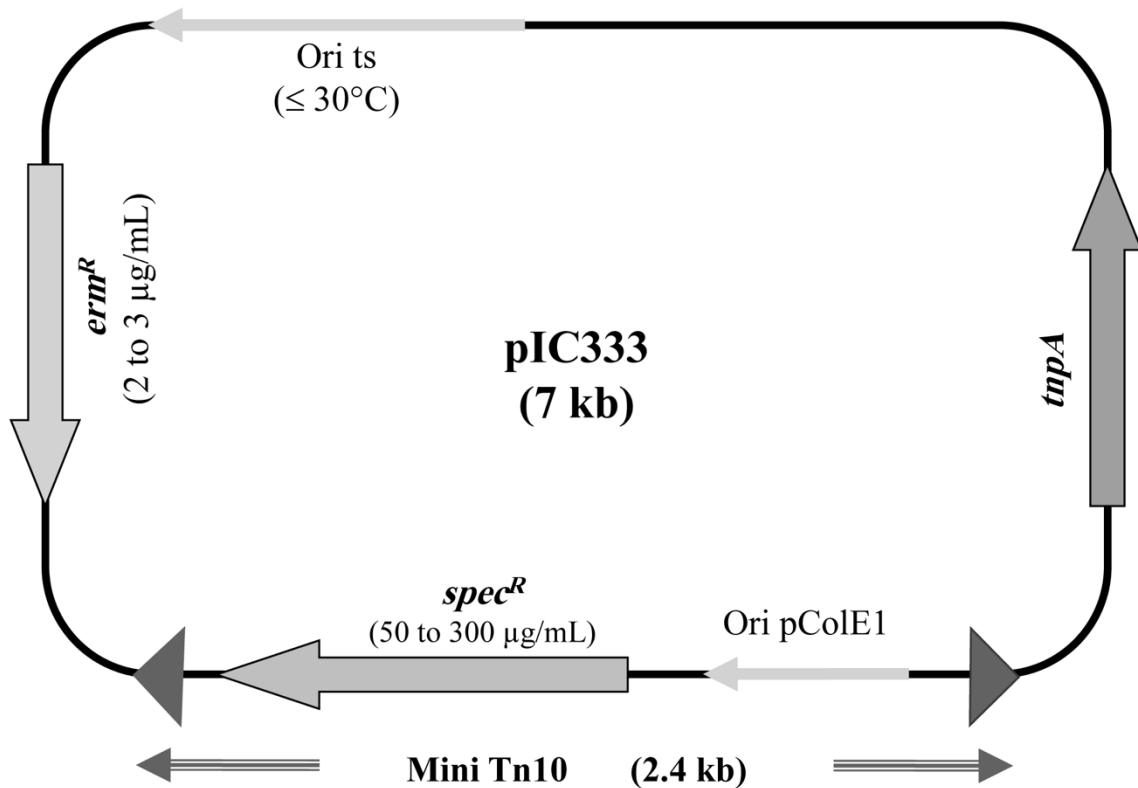
(A)



(B)



Supplementary Figure 1. The loss of *Bacillus thuringiensis* flagellar filaments enhances the release of immunostimulatory fragments of peptidoglycan. (A) Wild-type (wt) and *relish* (Rel) mutant flies were injected with the supernatant of *Bt407*, *Bt407*Δ*fliK*, *Bt407*Δ*fliK*Ω*fliK* or *Bt407*Δ*fla* cultures. Relative *Diptericin* (*Dipt*) expression was measured by RT-qPCR 4 h upon the induction. Ribosomal protein 49 (Rp49) transcript was used as reference gene. Transcripts levels are compared to that triggered in wt flies injected with Muller Hinton broth (MH) as a control. (B) Autolysis rates of *Bt407*, *Bt407*Δ*fliK*, *Bt407*Δ*fliK*Ω*fliK* and *Bt407*Δ*fla* in the presence of Triton X-100. Autolysis rates were determined over a period of 3 h (180 min) in PBS at 37 °C, without shaking. Data obtained from three independent experiments are combined in single value (mean ± sd). Statistical tests were performed using the Mann-Whitney test within Prism software (ns: $p > 0.05$; ****: $p < 0.0001$).



Supplementary Figure 2. Map of the temperature sensitive plasmid pIC333. The plasmid pIC333 carries the Gram-positive thermosensitive replication origin of pE194 plasmid (Ori ts), a gene conferring resistance to erythromycin (*erm^R*), a Tn10 transposase gene (*tnpA*) and the transposable element mini-Tn10. The mini-Tn10 (double grey arrow) is a 2.4 kb transposon flanked by inverted terminal repeat ends (dark grey triangles), containing a pColE1-type origin of replication (Ori pColE1) and a gene coding for resistance to spectinomycin (*spec^R*). This transposon system was originally used to generate random *B. subtilis* mutants (Steinmetz & Richter, 1994).

Supplementary Table 2. Primers sequences used in this study.

Name	Nucleotide sequence (5'→3')	Restriction site
fliK-a	CGCGGATCCGAACGTGGTCGGGAAGTAAA	BamHI
fliK-b	CCAACGTGTTGGCAGCCTTTATTCTCCAATATCATCCATTCATACACATTTCAAATCCAG	None
fliK-c	CTGGATTGAAAATGTGTATGAATGGATGATATTGGAGGAATAAAGCGTGCCAACAGTTGG	None
fliK-d	CATGCCATGGCGCATTGTCCATGTATTCCC	NcoI
CompfliK-a	CGCGGATCCGATGATATCGTTGAAGCGATACACC	BamHI
CompfliK-b	GGTAATACAGACTGTATCACCTGTTTTTCATAGCCCCCTTC	None
CompfliK-c	GGAAGGGGCTATGAAAACAGGTGATACAGTCTGTATTACC	None
CompfliK-d	CCGGAATTCTTATTCTCCAATAATCCCGC	EcoRI
E1	CGTTGGCCGATTCATTAATGC	None
E3	CGATATTCACGGTTTACCCAC	None
PU	CGCCAGGGTTTTCCAGTCACGAC	None
PR	AGCGGATAACAATTCACACAGGA	None
DiptRTFw	GCTGCGCAATCGCTTCTACT	None
DiptRTRv	TGGTGGAGTGGGCTTCATG	None
Rp49RTFw	GACGCTTCAAGGGACAGTATCTG	None
Rp49RTRv	AAACGCGGTTCTGCATGAG	None