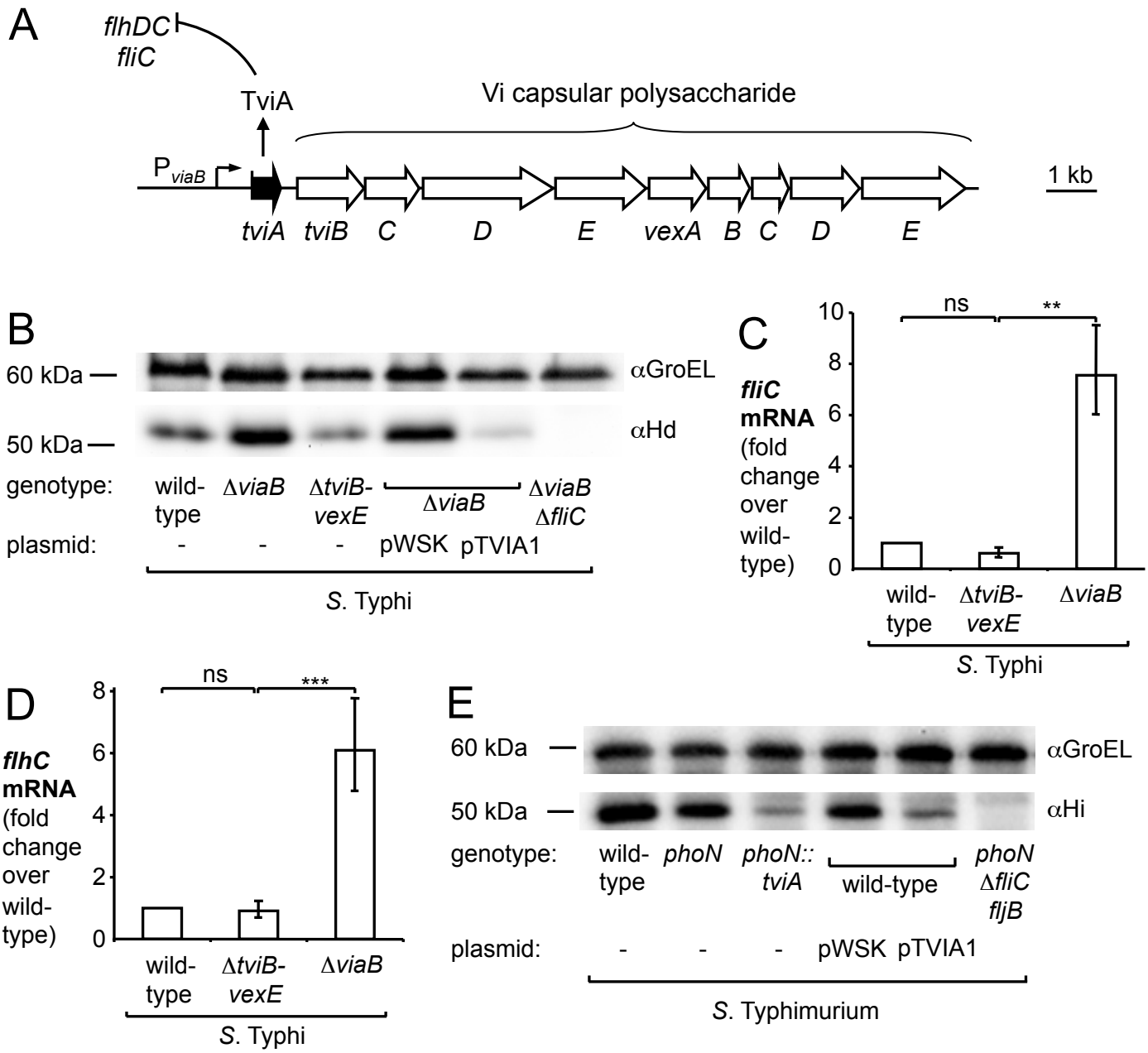

Figure S1: TviA represses expression of *fliC in vitro*. (A) Genetic organization and transcriptional regulation of the *viaB* operon of *S*. Typhi Ty2. (A) Expression of flagellin detected by Western Blot. The *S*. Typhi wild-type strain (Ty2), a Δ*viaB* mutant (SW347), a Δ*tviB-vexE* mutant (SW74), a Δ*viaB* mutant harboring the cloning plasmid pWSK29 (pWSK), a Δ*viaB* mutant expressing *tviA* from a plasmid (pTVIA1), and a Δ*viaB* Δ*fliC* mutant (SW483) were cultured in TYE broth. Expression of flagellin and GroEL was visualized by Western Blot using specific antiserum (αHd and αGroEL, respectively). The approximate location of standard proteins is indicated on the left. (C and D) Quantification of *fliC* (C) and *flhC* (D) mRNA levels. The *S*. Typhi strains were cultured in TYE broth. RNA was purified and relative gene expression normalized to the house keeping gene *gmk* determined by quantitative real time PCR (N = 4). (E) The *S*. Typhimurium wild type (IR715), a *phoN* mutant (AJB715), a *phoN*::*tviA* mutant (SW474), a wild-type strain harboring the cloning plasmid pWSK29 (pWSK), a wild-type strain expressing *tviA* from a plasmid (pTVIA1), and a *phoN* Δ*fliC fljB* mutant were cultured in TYE broth. Flagellin and GroEL levels were detected by Western Blot using specific antiserum (αHi and αGroEL, respectively).
Bars represent geometric means +/- standard error. **, $P < 0.01$; ***, $P < 0.001$; ns, not statistically significant; ND, none detected.

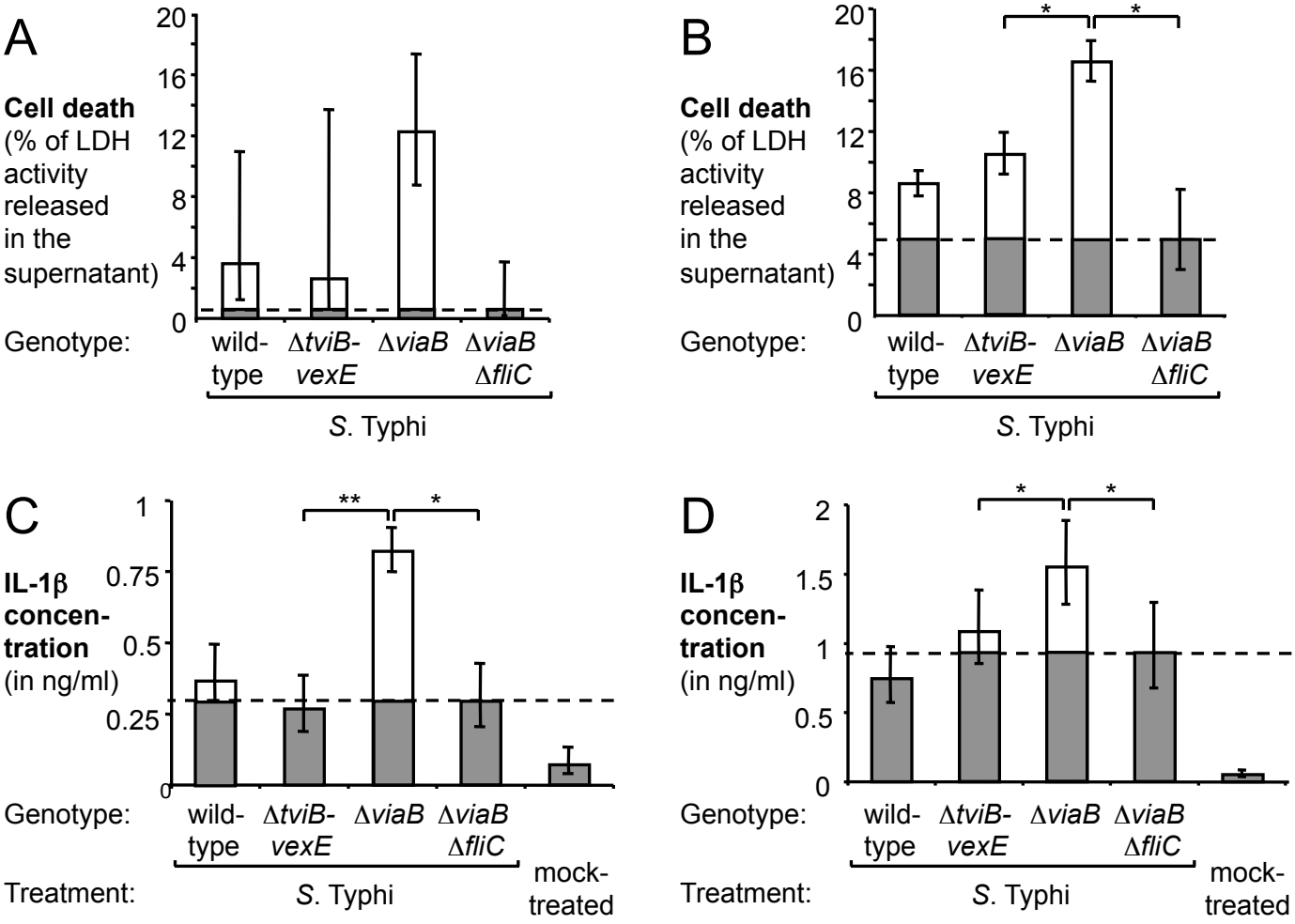

Figure S2: TviA reduces flagellin-mediated pyroptosis in *S.* Typhi-infected THP-1 cells. Differentiated THP-1 cells were mock-treated or infected with the following *S*. Typhi strains pre-cultured in TYE broth: wild type (Ty2), a Δ*viaB* mutant (SW347), a Δ*tviB-vexE* mutant (SW74), and a Δ*viaB* Δ*fliC* mutant (SW483). (A and B) Cell death after 4h (A) and 16 h (B) was determined using a lactate dehydrogenase (LDH) release assay. (C and D) IL-1β secretion into the supernatant after 4h (C) and 16 h (D) was measured by ELISA. FliC-dependent cell death and IL-1β release is indicated by a white color of the bar while LDH release and IL-1β secretion independent of FliC (i.e. amount induced by a Δ*viaB* Δ*fliC* mutant; dashed line) is indicated by the grey color of the bar. N = 5 (B and D) and N = 6 (C and E). Bars represent geometric means +/- standard error. *, $P < 0.001$; **, $P < 0.01$.

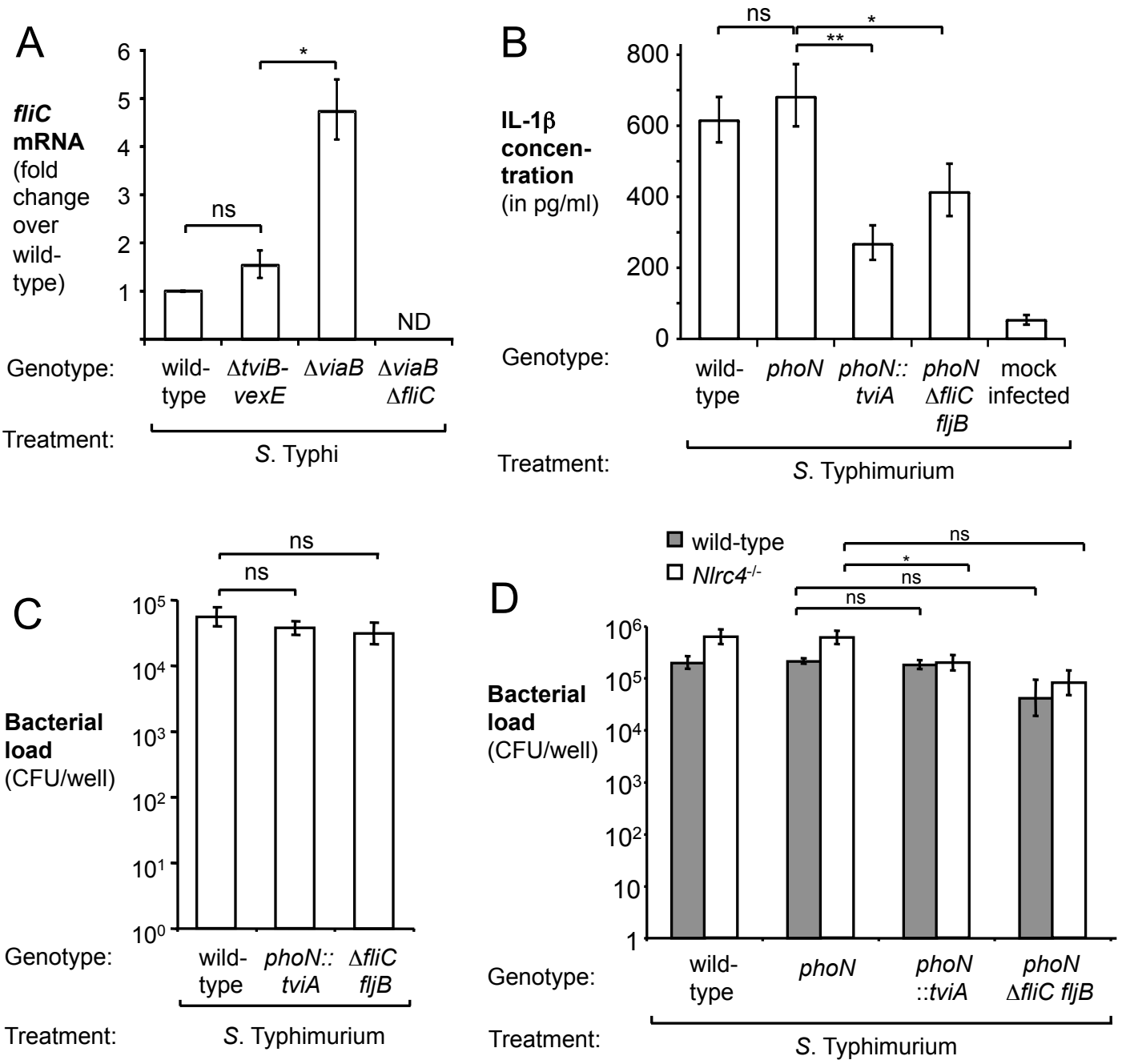

Figure S3: (A) The indicated *S*. Typhi strains were cultured in DMEM tissue culture media prior to infection of THP-1 cells. Total RNA was extracted 3 h after infection and *fliC* mRNA levels determined by real time PCR (N = 4). (B) THP-1 cells were infected with the indicated *S*. Typhimurium strains pre-cultured in TYE media. The concentration of IL-1β in the supernatant was quantified 8 h after infection (N = 4). (C) Bone marrow-derived macrophages were infected with the indicated *S*. Typhimurium strains and the amount of intracellular bacteria at the end of the experiment determined by plating (N = 3; see Fig. 3A). (D) Immortalized bone marrow-derived macrophages obtained from wild-type mice (black bars) or *Nlrc4*-deficient mice (white bars) were infected with the *S*. Typhimurium strains listed below the panel and the bacterial load determined (N = 3; see Fig. 4B).

Bars represent geometric means +/- standard error. *, $P < 0.05$; **, $P < 0.01$; ns, not statistically significant; ND, none detected