Supplemental Information for Publication: Guerreiro *et al.*, 2020

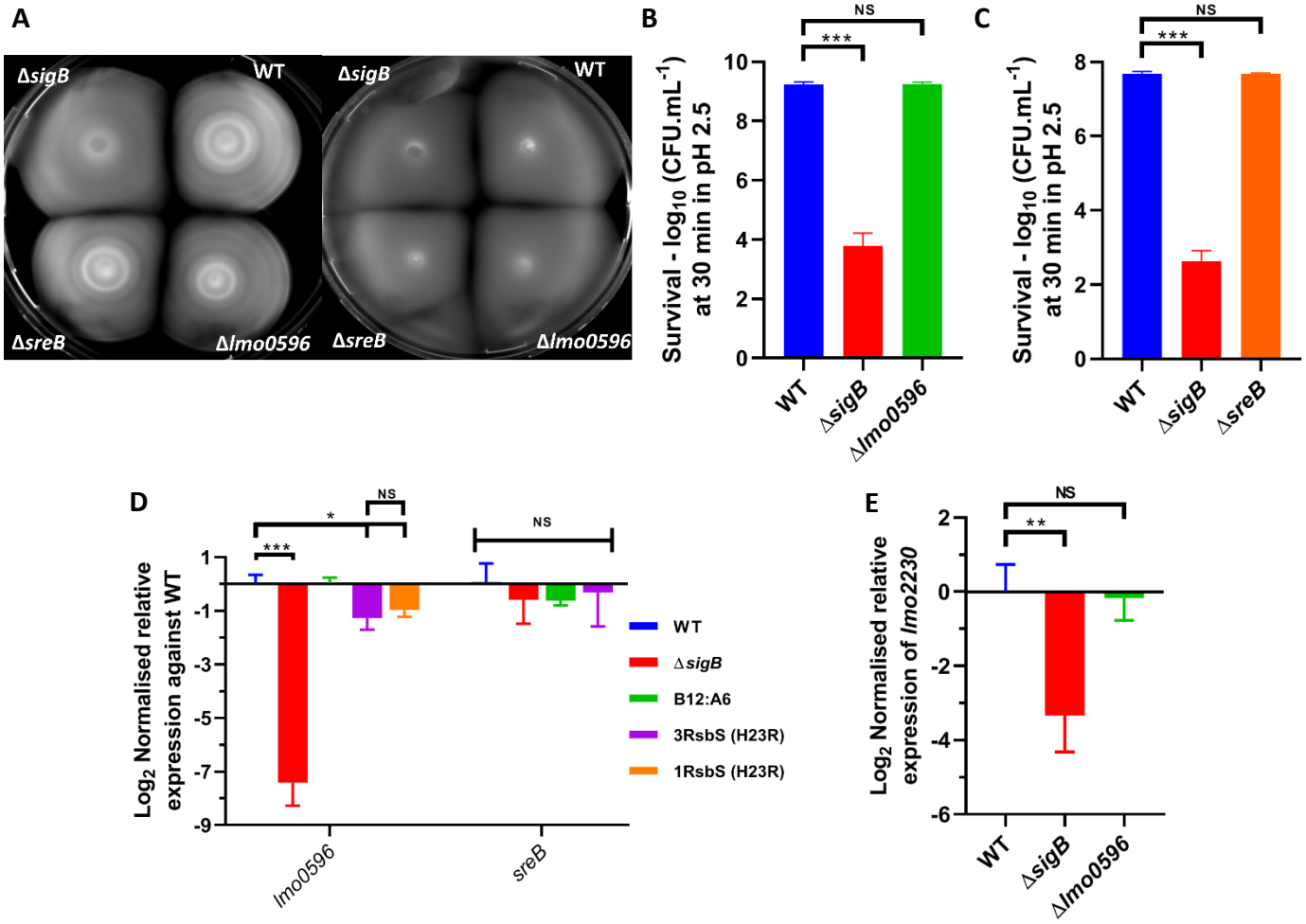

FIG S1 The transposon insertion in 3RsbS (H23R) does not influence σ^{B}-dependent phenotypes. (A) Ring formation phenotype of WT, Δ*sigB*, Δ*lmo0596* and Δ*sreB* mutant strains grown in BHI soft agar plates (left) exposed to cycles of light and dark and (right) constant dark. (B and C) Survival of stationary phase cells grown at 37°C of WT, Δ*sigB* Δ*lmo0596* and Δ*sreB* after 30 min of exposure to acidified BHI (pH 2.5). (D) Transcript levels of *lmo0596* and *sreB* relative to the WT strain in mid-log phase cells (OD_{600} = 0.8) of WT, Δ*sigB*, B12:A6, 3RsbS (H23R) and 1RsbS (H23R) mutant strains. A minimum of three biological replicates was performed for both acid survival and gene expression measurements. (E) Expression of the σ^{B}-dependent gene *lmo2230* relative to the WT strain in mid-log phase cells (OD_{600} = 0.8) of WT, Δ*sigB* and Δ*lmo0596* mutant strains. Statistical analysis was performed using a paired Student *t*-test always compared against the WT strains (* = *p*-value < 0.05; ** = *p*-value < 0.01; *** = *p*-value < 0.001; NS – non-significant).

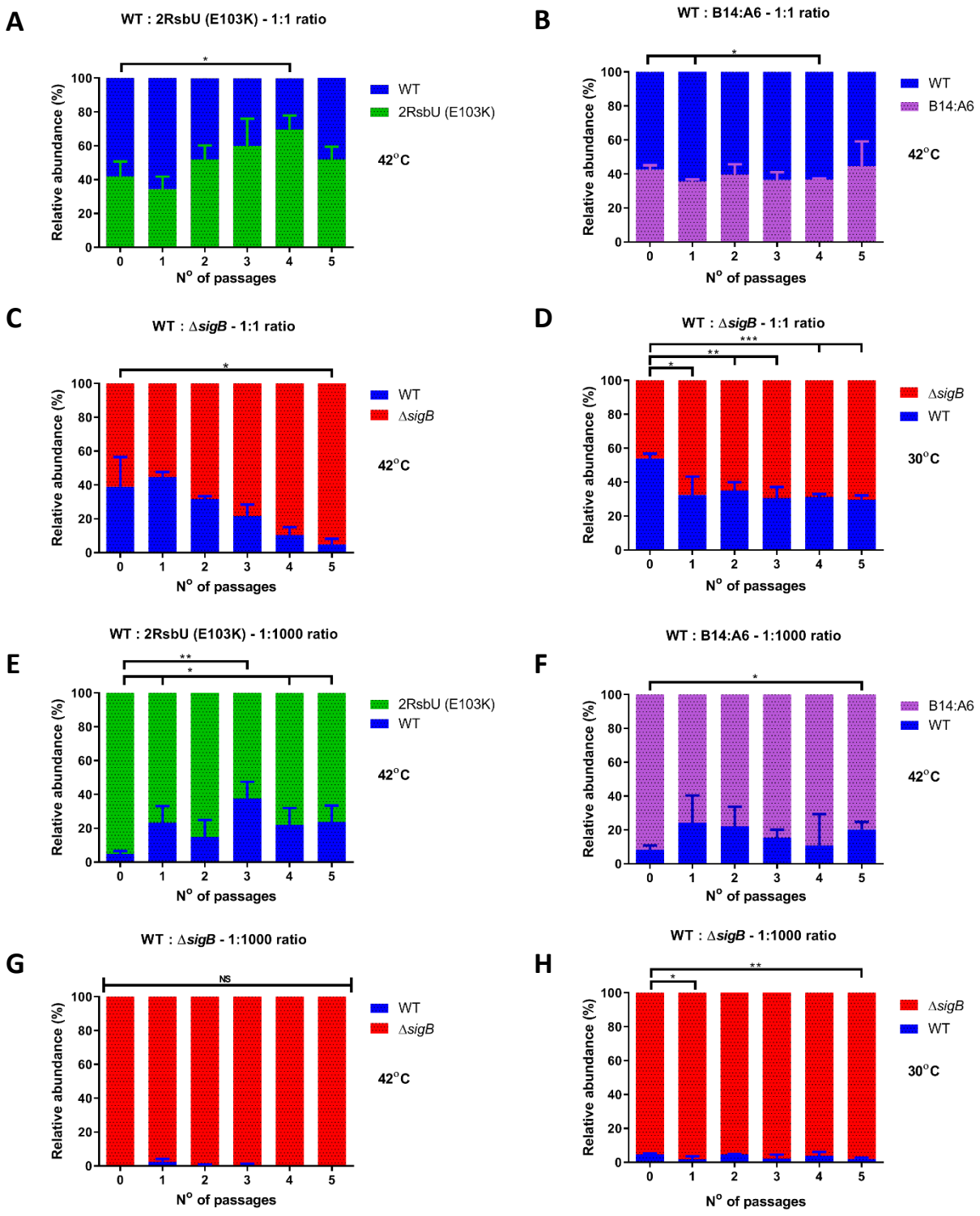

FIG S2 Competitive advantage of Δ*sigB* mutant strain relative to WT strains is less evident at 30°C. Competition experiments of mixed cultures of WT, Δ*sigB*, B14:A6 and 2RsbU (E103K) mutant strain showing the relative abundance in percentage of CFU.mL^{-1}. Cultures incubated at 42°C were mixed in ratios of 1:1 of (A) WT with 2RsbU (E103K), (B) WT with B14:A6, (C) WT with Δ*sigB* and 1000:1 of the respective (E), (F) and (G). WT and Δ*sigB* were incubated at 30°C in ratios of (D) 1:1 and (H) 1000:1. Passages were made every 24 hours for 5 days. Data generated from three independent biological replicates. Statistical analysis was performed using a paired Student *t*-test (* = *p*-value < 0.05; ** = *p*-value < 0.01; *** = *p*-value < 0.001).

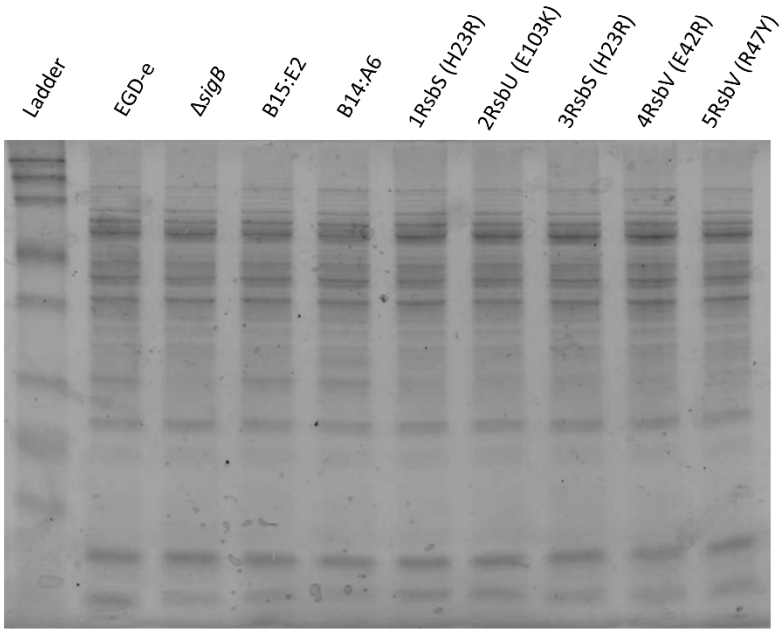

FIG S3 SDS-PAGE of normalized total protein extract used for all western-blots in this study. SDS-PAGE image from total protein extractions of stationary phase cultures of WT, Δ*sigB*, and transposon mutant strains grown at 37°C. Total protein fractions were normalized to 0.8 mg.mL^{-1} of total protein separated in 15% acrylamide/bis-acrylamide gels along with PageRuler™ Plus Prestained ladder. Gels were later stained with GelCode® Blue Staining Reagent and destained in destaining solution.