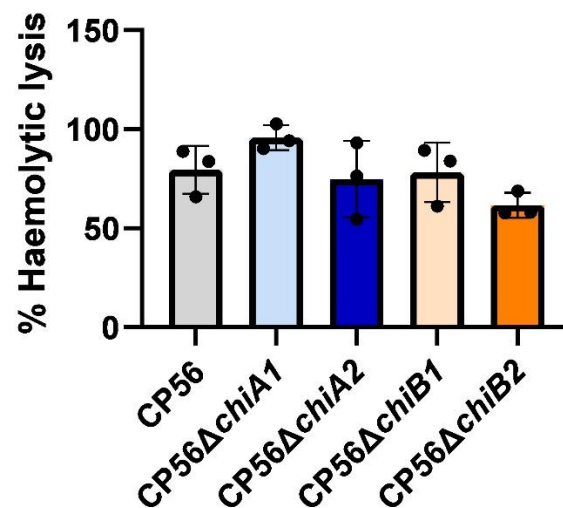


**S4 Fig: NetB activity in culture supernatant of *C. perfringens* strains (wild-type and mutant strain) towards chickens erythrocytes.**

The NetB production of the *C. perfringens* strains was evaluated through an haemolytic assay, previously described by Hustá *et al.* In short, 20% culture supernatant was incubated with 2% (v/v) chicken erythrocytes (diluted in HBSS-) in a 96-well plate. After an incubation period of 30 minutes at 37°C, intact red blood cells were pelleted and the released hemoglobin in the supernatant was quantified at OD<sub>550nm</sub>. A matched non-parametric Friedman test with Dunn' multiple comparison (confidence interval 95%) was performed using GraphPad Prism 8 software. Bars indicate the means with their respective standard deviations. No significant difference in NetB activity between the strains could be detected.



[1] Hustá M, Ducatelle R, Van Immerseel F, Goossens E. A Rapid and Simple Assay Correlates In Vitro NetB Activity with *Clostridium perfringens* Pathogenicity in Chickens. *Microorganisms*. 2021 Aug 11;9(8):1708. doi: 10.3390/microorganisms9081708. PMID: 34442787; PMCID: PMC8400579.