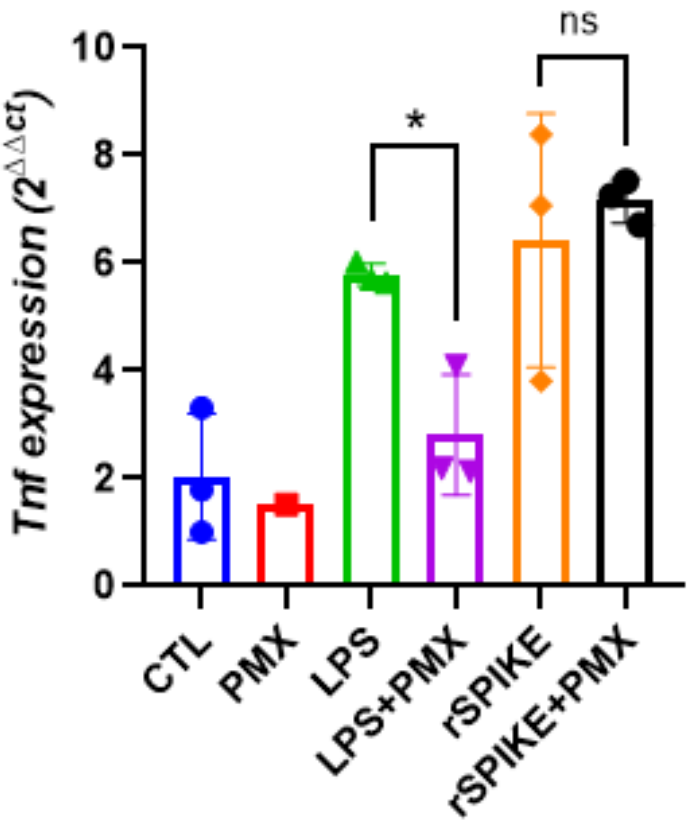
Photobiomodulation reduces the cytokine storm syndrome associated with Covid-19 in the zebrafish model

We confirm that our protein (rSpike) that there is no contamination by LPS.



For this, J774A.1 cell line were used to analyze the neutralization of LPS itself and possible LPS contamination of recombinant Spike protein (rSpike) produced in E. coli. J774A.1 cells were cultured in DMEM high glucose containing 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 30 mM HEPES. Cells were plated at a density of 5x105 cells/well in the presence or absence of Polymyxin (PMX) B (10 µg/mL, Sigma-Aldrich) and were stimulated with LPS (100 ng/mL, Sigma-Aldrich) or rSpike (2.5 µg/mL). Unstimulated cells in the presence or absence of PMX were cultured as a control. Cells were incubated at 37°C, 5% CO2, for 8 hours. Then, the cells were collected and submitted to qPCR analysis.

RT-PCR analysis

Total RNA was isolated by TRIzol Reagent methods (Invitrogen, USA) and total RNA concentrations were determined in Nanodrop. cDNA synthesis was performed using the

MML-V reverse transcriptase kit (Promega, USA), conforming to manufacturers' specifications. PCR amplification and cycles were run with QuantStudio 3 PCR Systems with SYBR green assays with a two-step PCR protocol (50ºC for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15s and 60°C for 1 min). For SYBR green assay, the validated oligo was used: TNF-alpha (Forward: 5'-GCCCCCAGTCTGTATCCTTCTAA-3' - Reverse: 5'-ACTGTCCCAGCATCTTGTGTTTC-3'). The gene expression was normalized by the relative expression of an internal control gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH, (Forward: 5'- TACTCGCGGCTTTACGGG -3' - Reverse: 5'-TGGAACAGGGAGGAGCAGAGAGCA -3'). The Ct (cycle threshold) was determined automatically for each sample and run in triplicate, considering that relative gene expression was calculated by the 2-ΔΔCT method.