Molecular Medicine

Supplemental Data

Development of a Zebrafish Sepsis Model for High-Throughput Drug Discovery

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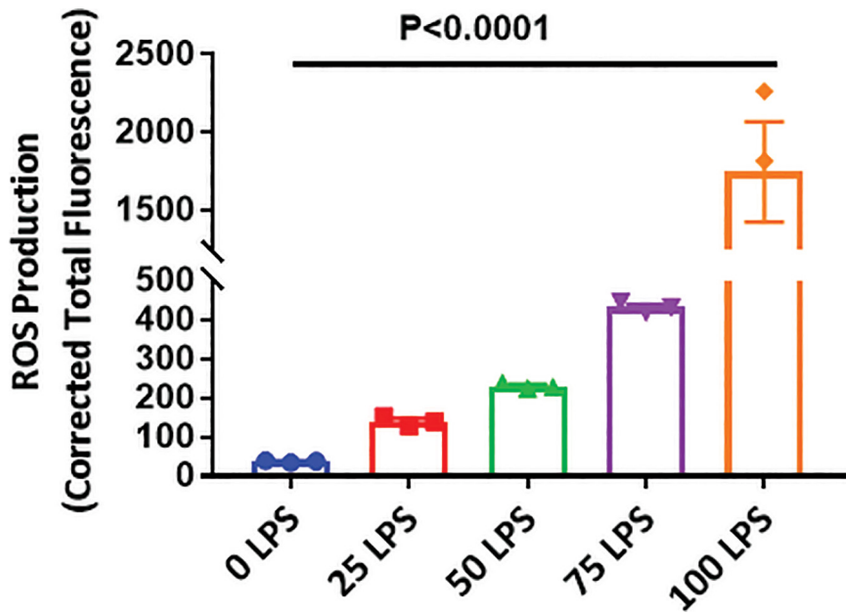

Supplementary Figure S1. LPS exposure (25, 50, 75 and 100 μg/mL) significantly increases ROS production in a dose-dependent manner (n = 3/treatment). ROS production was measured using a ROS indicator, namely 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA).

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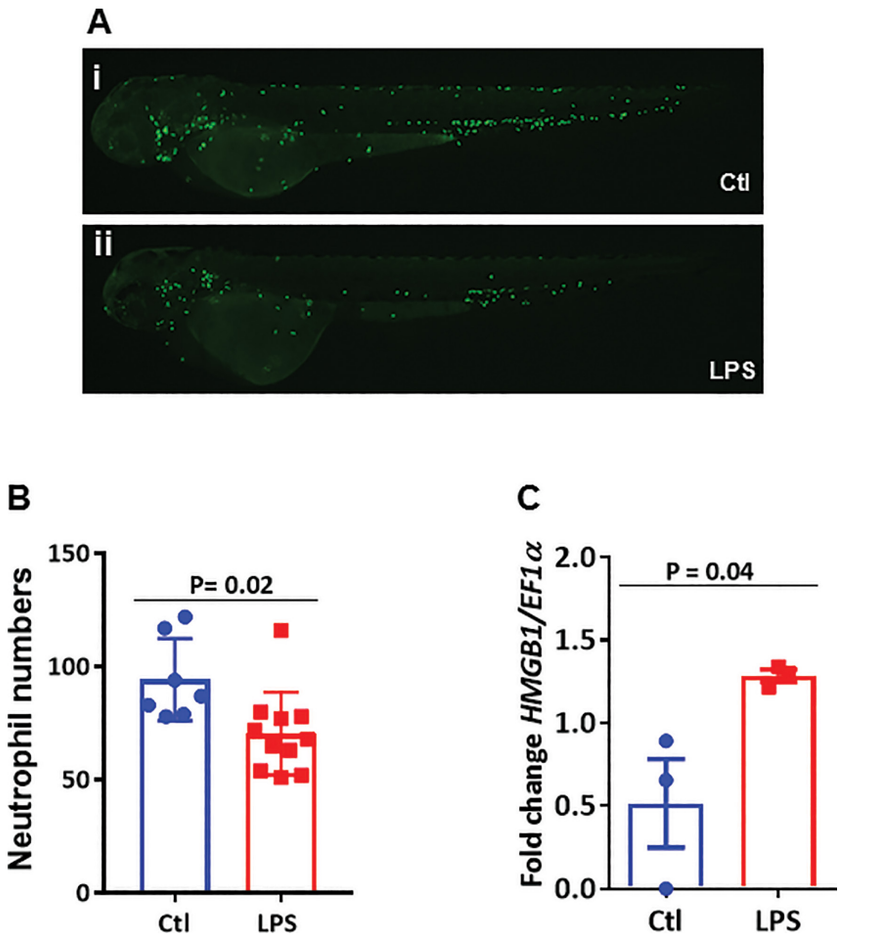

Supplementary Figure S2. LPS causes reduced neutrophil numbers and increased HMGB1 expression in the later stages of inflammation in the zebrafish sepsis model. Three DPF zebrafish larvae exposed to LPS (100 μg/mL) showed (A, B) reduced whole-body neutrophil numbers (n = 7–12/treatment) and (C) increased *HMGB1* transcript levels (an average of 3 trials, each with a pool of 20 larvae/treatment) at 6–7 h post LPS treatment when compared with controls.

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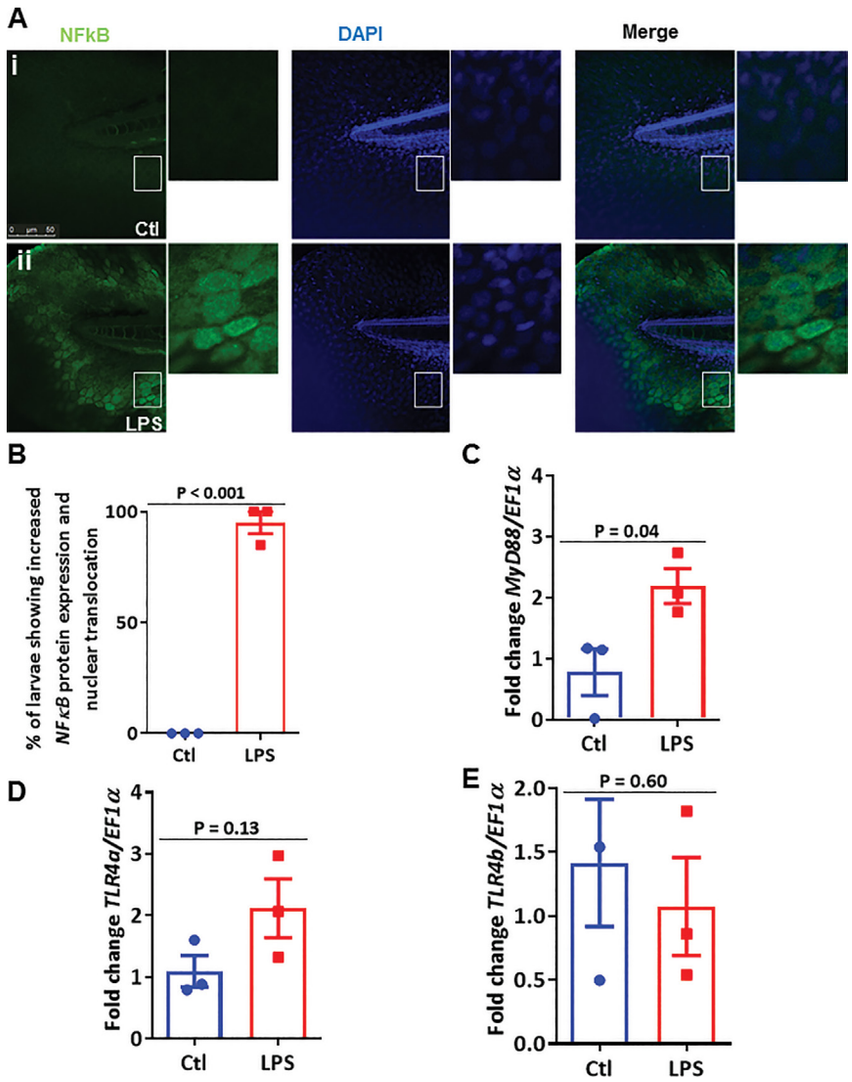

Supplementary Figure S3. LPS signaling in the zebrafish sepsis model involves increased NFκB protein synthesis, nuclear NFκB translocation and increased expression of MyD88. Three DPF zebrafish larvae were exposed to LPS for 2 h for immunofluorescence studies and 6 h for quantitative real-time PCR analysis. (A, B) Immunofluorescence microscopy of 3 DPF zebrafish larvae exposed to LPS for 2 h reveals synthesis of NFκB protein and its nuclear translocation (an average of 3 trials with 3–4 larvae/treatment). The larvae were stained with NFκB/p65 rabbit polyclonal primary antibody Alexa fluor 488 secondary antibody and DAPI (nuclear staining) as indicated in the Methods and Materials section. Fluorescence with anti-NFκB/p65 Alexa fluor 488 antibodies and with DAPI and merged fields between the two are shown. Exposure of 3 DPF zebrafish larvae to LPS for 6 h resulted in (C) increased *MyD88* transcript levels, but (D, E) no significant changes in the transcript levels of *TLR4a* and *TLR4b* (an average of 3 trials, each with a pool of 20 larvae/treatment).

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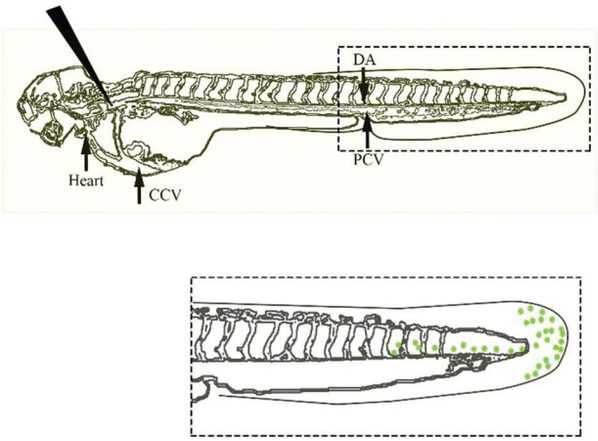

Supplementary Movie S1. Time-lapse imaging of LPS-induced vascular leakage through the intersegmental vessels (ISVs). Three DPF zebrafish larvae were exposed to vehicle (top) or LPS (bottom) for 6 h and then injected with FITC-dextran to observe vascular leakage in real time. In the LPS-treated larvae, we can see FITC-dextran leaking out through the last few ISVs, moving toward the tail fin and accumulating at the tip of the tail fin over time. A schematic representation of what is shown in the movie is given above. FITC-dextran is injected into the CCV of zebrafish larvae exposed to vehicle or LPS. In the LPS-treated larvae, we can see the FITC-dextran leaking out through the last few ISVs, moving toward the tail fin and accumulating at the tip of the tail fin over time.

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