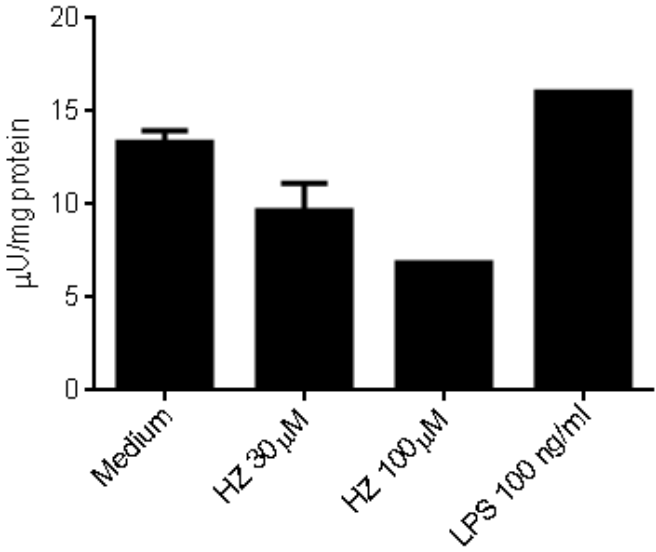


Additional file 2_Figure



Additional file 2_Legend

Measurement of urea levels in BMDM treated with malarial hemozoin.

BMDM were seeded (24-well plates; 2.5×10^5 cells/well, in 500 μ l of experiment medium) at 37°C under an atmosphere of 5% CO₂, overnight. Cells were primed with 12.5 U/ml IFN- γ for 2h, and then stimulated with different concentrations of HZ (30, 100 μ M), or LPS (200 ng/ml). After 24h incubation, the cells were washed with PBS and lysed in a cell lysis buffer (Cell Signaling Technology®). The concentration of protein of each sample was determined through Bradford reagent (Biorad®) ².

The cell lysate was incubated with 0,25 M L-Arg (pH 9,7) for 30 min at 37°C.

The reaction was stopped by adding an acid mixture (H₂SO₄-H₃PO₄-H₂O, 1:3:7, vol/vol/vol). Arginase activity in the samples was reflected as the quantity of urea formed, which was expressed as the μ mol of urea produced per min (μ U) per milligram of protein. The urea levels were measured at 540 nm in a spectrophotometer (Synergy 4 microplate reader, Biotek, GE) ³. Data shown as averages \pm SD of three independent experiments.