Supplemental Material

Oxidative Stress Induces Expression of the Toll-Like Receptors (TLRs) 2 and 4 in the Human Peripheral Blood Mononuclear Cells: Implications for Metabolic Inflammation

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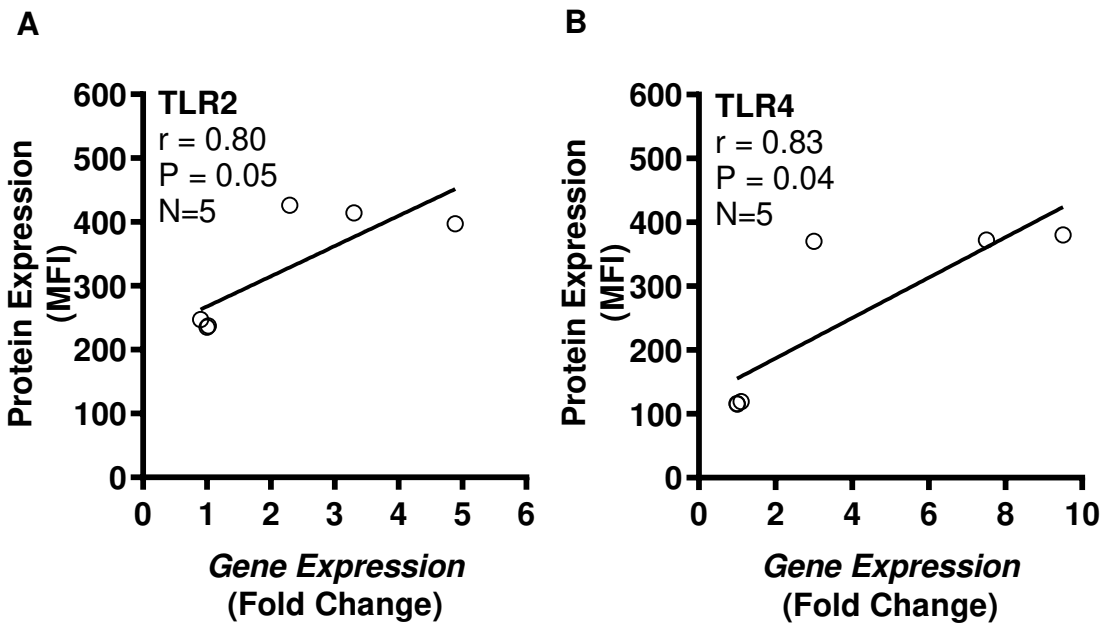
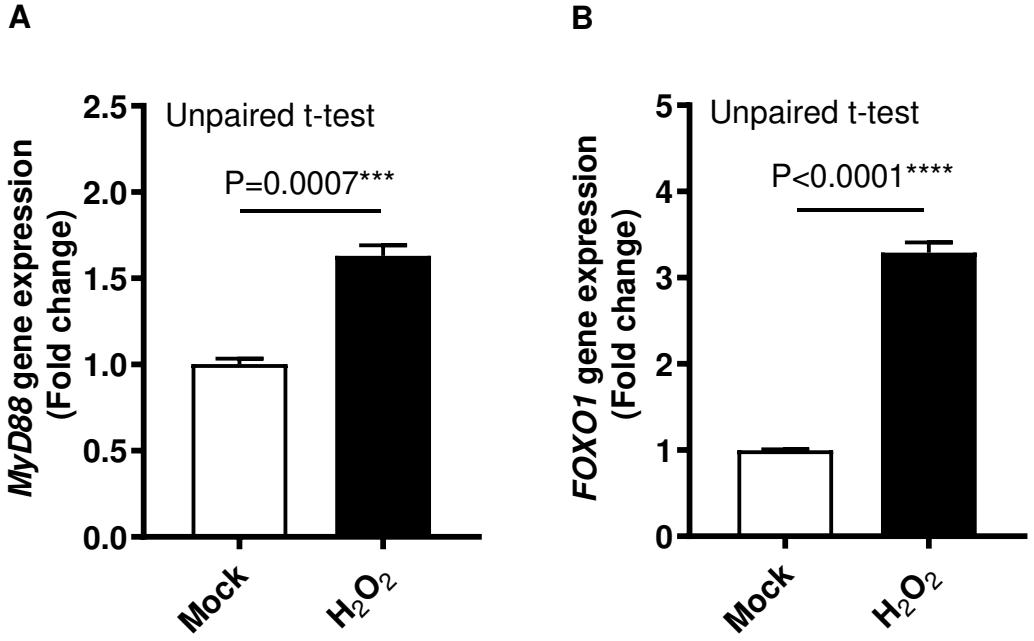


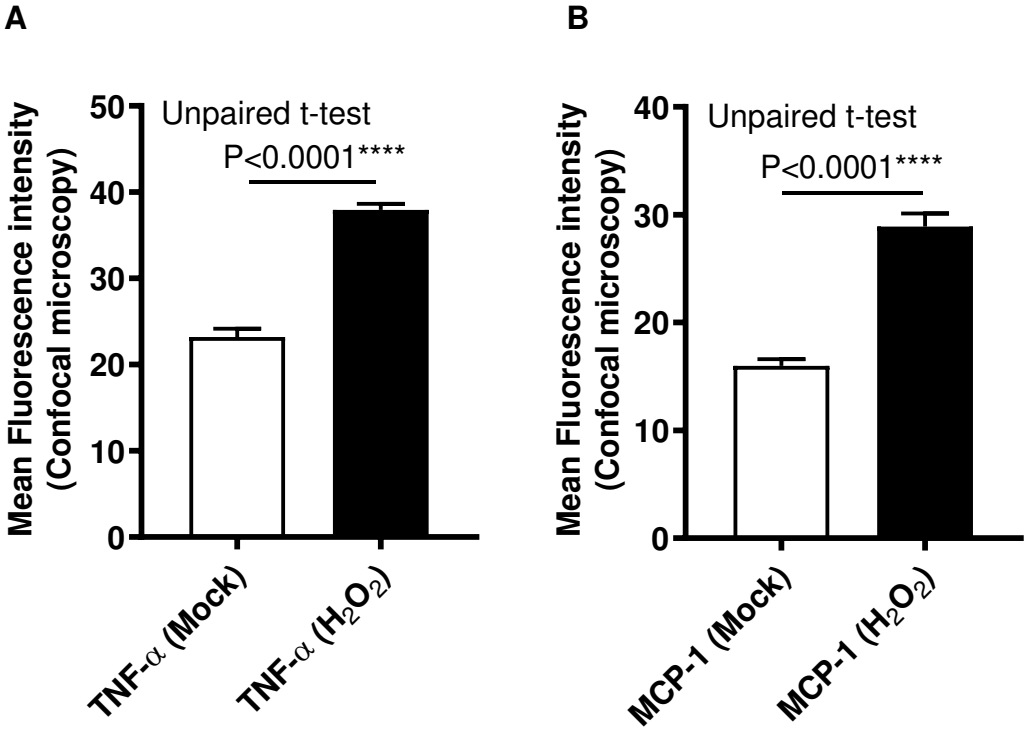
Peripheral blood mononuclear cells (PBMC) isolated from healthy individuals were cultured in triplicate wells (10^6 cells/mL/well) of 12-well plates and incubated for 10h with H_2O_2 (10mM) or vehicle (mock). Total RNA was extracted from harvested cells using RNeasy kit following the manufacturer's instructions. TLR2/4 gene expression (Fold Change) was assessed using real-time RT-PCR as described in materials and methods. At the same time, harvested samples were also stained to determine TLR2/4 receptor surface expression (Mean Fluorescence Intensity) by flow cytometry. The data (mean±SEM) obtained from 5 independent determinations with similar results show a good agreement between gene and protein expression of (**A**) TLR2 (r=0.80 P=0.05) and (**B**) TLR4 (r=0.83 P=0.04).

Supplementary Figure S1



Peripheral blood mononuclear cells (PBMC) isolated from 5 healthy individuals were cultured in triplicate wells (10^6 cells/mL/well) of 12-well plates and incubated for 10h with H_2O_2 (10mM) or vehicle only (mock). Total RNA was extracted from harvested cells using RNeasy kit following the manufacturer's instructions. MyD88 and FOXO1 gene expression was determined using real-time RT-PCR protocol as described in materials and methods and the following primers (MyD88: Hs01573837_g1; FOXO1: Hs00231106_m1). The data (mean±SEM) obtained from 5 independent determinations with similar results show significantly increased expression of (**A**) MyD88 and (**B**) FOXO1 in PBMC treated with H_2O_2 (MyD88: 1.63±0.06 fold; FOXO1: 3.29±0.12 fold) compared to mock (MyD88: 1.0±0.03 fold; FOXO1: 1.0±0.01 fold).

Supplementary Figure S2



Peripheral blood mononuclear cells (PBMC) isolated from healthy individuals were cultured in triplicate wells (10^6 cells/mL/well) of 12-well plates and incubated for 10h with H_2O_2 (10mM) or vehicle (mock). TNF-α and MCP-1 protein expression (shown as MFI) was detected by confocal microscopy as described in materials and methods. The data (mean±SEM) obtained from 2 independent determinations with similar results show significantly elevated expression of (**A**) TNF-α and (**B**) MCP-1 in PBMC treated with H_2O_2 (TNF-α: 37.94±0.69; MCP-1: 28.89±1.24) compared to mock (TNF-α: 23.20±0.95; MCP-1: 15.99±0.61).

Supplementary Figure S3