Online Appendix

Methods: ***Reagents***

THP-1 human monocytic cell line was obtained from American Type Culture Collection (Manassas, VA). Endotoxin-free, glucose-free RPMI-1640 media and fetal bovine serum (FBS) were purchased from Gibco BRL (Carlsbad, CA). Antibiotics, glutamine, phenylmethylsulfonyl fluoride, endotoxin-free D-glucose/mannitol, HEPES, protease inhibitor cocktail, Triton X-100, dithiothreitol (DTT) were from Sigma Chemical (St. Louis, MO). Calphostin-C (5 & 10μmol/l), chelerythrine (2.5 & 5μmol/l), GF109203X (5 & 10μmol/l), Apocynin (15, 30μmol/l), and diphenyleneiodonium chloride (DPI, 5 &10μmol/l), were obtained from Calbiochem. Purified LPS was obtained from List Biological Laboratory Inc. A synthetic bacterial lipoprotein (PamCAG: palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)propyl)-Ala-Gly-OH) was purchased from Bachem (King of Prussia, PA). Poly(I:C) was purchased from Amersham Biosciences (Piscataway, NJ). Macrophage-activating lipopeptide, 2 kDa (MALP-2) was purchased from Alexis Biochemical (San Diego, CA). Mouse pDisplay-HA-TLR4, 2, 1 and 6 were obtained from Lynn Hajjar (University of Washington). NF-κB (2x)-luciferase reporter construct was provided by Frank Mercurio (Signal Pharmaceuticals, San Diego, CA). pRSV-β-galactosidase plasmid was from Jongdae Lee (University of California, San Diego, CA). Anti-human TLR2 (#17-922) and TLR4 antibodies (#12-9917) or isotype matched IgG controls (mouse IgG2a, #14-4724) were obtained from eBioscience, San Diego, CA. Interleukin receptor associated Kinase-1 (IRAK-1), MyD88, total IRAK-1, and anti-rabbit or anti-mouse IgG-horseradish protein were from Cell Signaling, MA. Pre-validated Protein Kinase C-α (Genbank# NM_002737), Protein kinase C-β (Genbank # NM_002738), Protein Kinase C-δ (GenBank#NM_212539.1), and p47Phox (Genbank# NM_000265), TLR2 (GenBank# NM_003264), and TLR4 (Genbank# NM_138554) siRNAs and siPORT amine reagent were purchased from Ambion (Austin, TX). The concentrations of the various inhibitors and siRNAs used in the present study were cautiously titrated and determined in pilot studies and were reported previously (15, 30, 32).

***siRNA Transfection assays*:** Pre-validated small interfering RNAs (siRNA) were obtained from Ambion and transfection assays were performed as described previously (32) following manufacturer's instructions, with suitable vehicle and scrambled siRNA controls, and subsequently treated with HG (15mM) for 24hrs. Transfection rates of 70-80% of cells were accepted for all the experiments. Knock-down efficiency of the siRNAs is indicated via i) flow cytometric analysis of TLR2 and TLR4, ii) real time RT-PCR of the target gene, and iii) measuring the end product (cytokines) of the target gene.

***NF-κB transcription factor Assay*:** NF-κB p65 DNA binding activity in the HG treated nuclear extracts of monocytes was determined using the nonradioactive TransAM transcription factor assay® (Active Motif, Carlsbad, CA) as described in detail (30) following manufacturer's instructions. The intra and inter assay CV for these assays was < 7%.

***PKC activity assays*:** PKC activity in monocytes was determined by non-radioactive immunoassay (Stressgen, Ann Arbor, MI) effectively same as previously described (30) following manufacturer's instructions. Relative PKC activity in the cell lysates is calculated and expresseas Absorbance/mg protein. The intra and inter assay CV was < 10%.

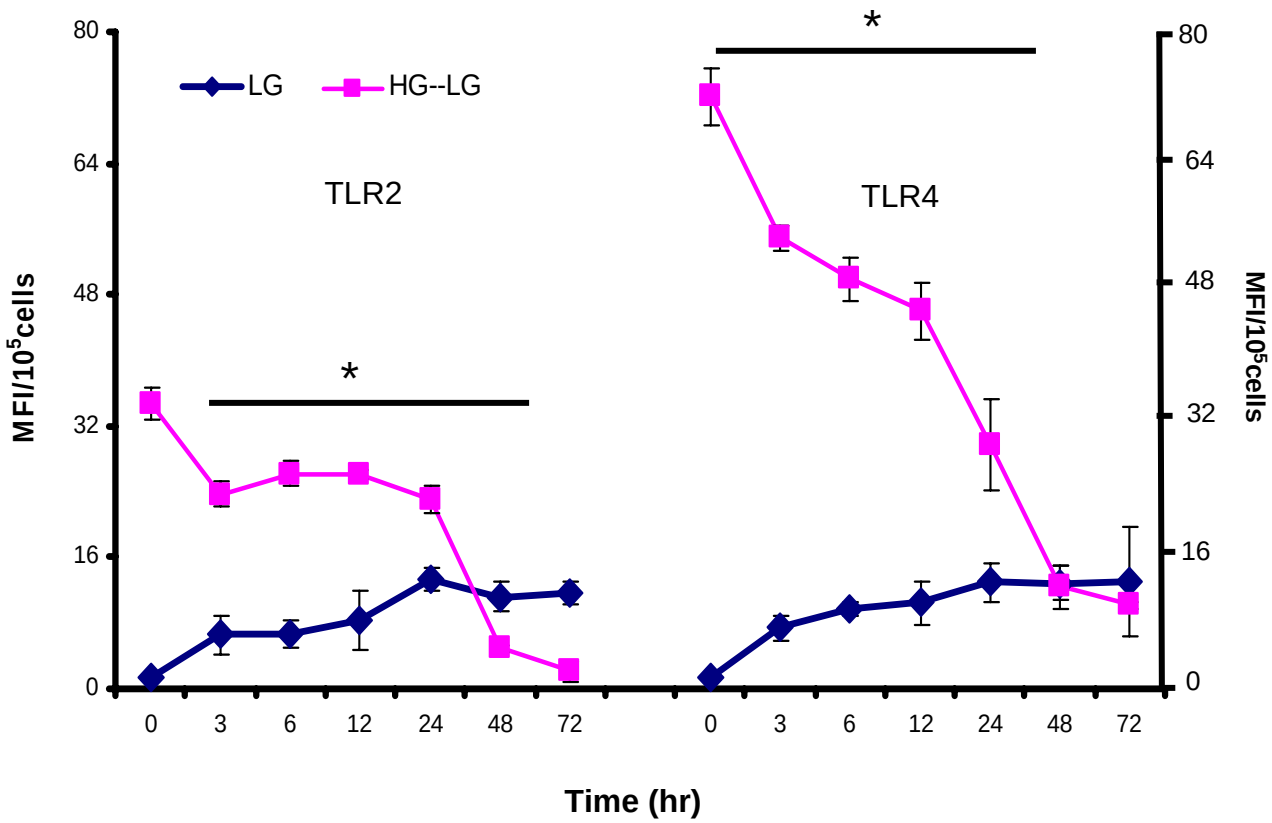
Human Toll like receptor pathway specific RT^2 Profiler^{TM} PCR Array analysis: Quantitative RT-PCR was performed using manufacturer's protocols for the (Human Toll like receptor Pathway Superarray, Gaithersburg, MD). A minimum of three array plates were used per treatment. Relative gene expressions were calculated by using the $2^{-\Delta\Delta Ct}$ method and normalized ΔCt value of each sample (31) using a total of five endogenous control genes (B2M, *HPRT1*, *RPL13A*, *GAPDH*, and *ACTB*). Fold change values are then presented as average fold change = $2^{-(\text{average } \Delta\Delta Ct)}$ for genes in treated relative to control samples.

Appendix Table 1. Up regulated TLR pathway genes in high glucose treated THP-1 monocytic cells compared to 5.5mM glucose.

Gene name	Gene symbol	Fold change	P value
Toll like receptor-4	TLR4	364.5	8.99E-06
TANK binding kinase-1	TBK1	88.2	5.47E-05
Interleukin-1 receptor associated kinase-1	IRAK1	58.7	5.54E-05
Tumor necrosis factor	TNF	19.9	0.00041
Toll like receptor-2	TLR2	48.6	0.000937
Myeloid differentiation factor 88	MYD88	24.4	0.001391
Nuclear factor-κ gene enhancer in Bcells inhibitor	NFKBIA	27.7	0.002593
Toll like receptor-7	TLR7	12.0	0.004228
Interleukin-1, beta	IL1B	8.0	0.005928
Bruton agammaglobulinemia tyrosine kinase	BTK	38.8	0.006463
Nuclear factor-κ gene enhancer in Bcells (p50/p105)	NFKB2	6.0	0.006847
Nuclear factor-κ gene enhancer in Bcells (p50/p65)	NFKB1	23.3	0.008725
TNF receptor associated factor 6	TRAF6	2.1	0.016014
Toll like receptor adaptor molecule 2	TICAM2	5.9	0.017876
Toll like receptor-6	TLR6	5.3	0.019813
Chemokine (C-X-C motif) ligand 10	CXCL10	17.6	0.024761
CD14 antigen	CD14	1.8	0.03063
Interferon regulatory factor 1	IRF1	8.0	0.048628
Toll like receptor 3	TLR3	4.5	0.049366
Interleukin 6	IL6	3.8	0.056191

Inhibitor of kappa light chain in B cells, kinase beta	IKBKB	4.8	0.504698
House Keeping genes			
Beta 2 microglobulin	B2M	-0.28	NS
Hypoxanthine phosphoribosyltransferase 1	HPRT1	-0.48	NS
Ribosomal Protein L13A	RPL13A	-0.27	NS
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	-0.42	NS
Actin-Beta	ACTB	-0.38	NS

Appendix Figure 1



THP-1 cells were challenged with HG for 24hr, transferred to 5mM glucose (LG), and TLR2 and TLR4 expressions were measured at indicated time points by Flow cytometry as described in Methods. Values are expressed as mean fluorescence intensity units (MFI)/10^5 cells ± SD. *P<0.05 vs LG alone; n = 4 experiments. HG=High glucose