Supporting Information

Plotnikov et al. 10.1073/pnas.1307096110

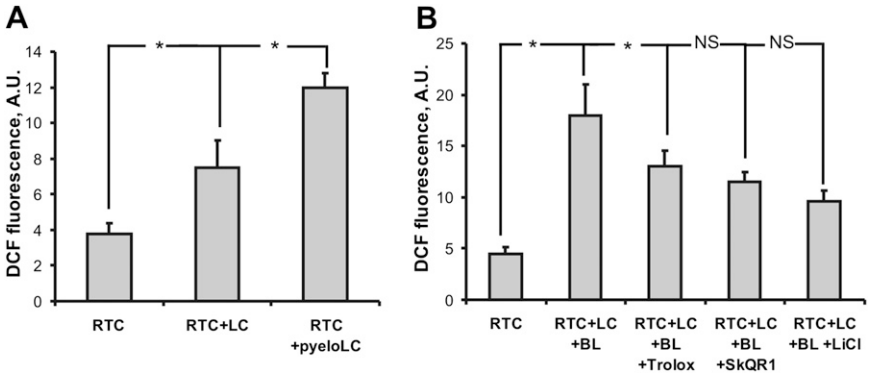

Fig. S1. Oxidative stress in a cellular model of pyelonephritis in vitro. Quantitative results from confocal microscopy of renal tubular cells (RTCs) stained with 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA). (*A*) After incubation with leukocytes (LC) from pyelonephritic rats (pyeloLC), DCF fluorescence rose up in higher extent, compared with intact rat LC. (*B*) Wash-out of antioxidant (1 h of incubation with antioxidant followed by three wash-outs with nutrient medium for 15 min each) before coculture with leukocytes does not abolish the protection. A.U., arbitrary units; BL, bacterial lysate; NS, not significant.

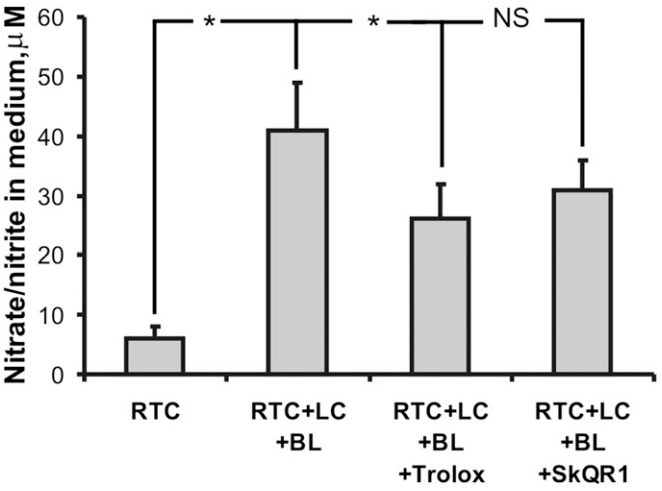

Fig. S2. Effect of the antioxidants on nitrite production by leukocytes (LC). BL, bacterial lysate; SkQR1, mitochondrial antioxidant, 10(6′-plastoquinonyl) decylrhodamine 19.

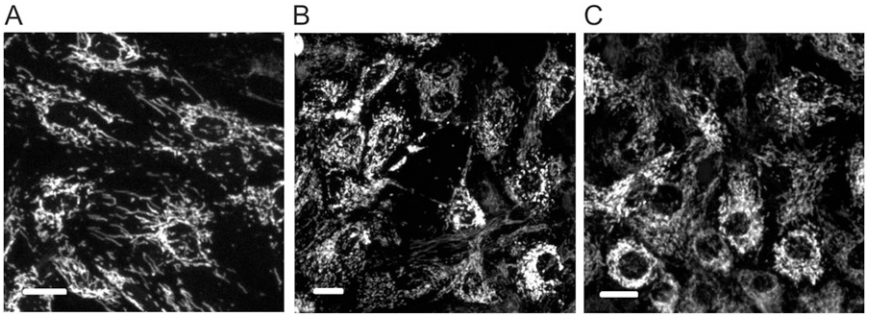

Fig. S3. Fragmentation of mitochondrial reticulum. (*A*) Control renal cells (note that the majority of mitochondria are presented by long filaments). (*B*) Renal cell after 24 h coculture with leukocytes and bacterial lysate (mitochondria are mostly fragmented). (*C*) As in *B* with addition of 5 mM nitro-L-arginine methyl ester (LNAME) during 24 h cocultivation (mitochondria partially restore their filamentous structure). (Scale bar, 20 μm.)

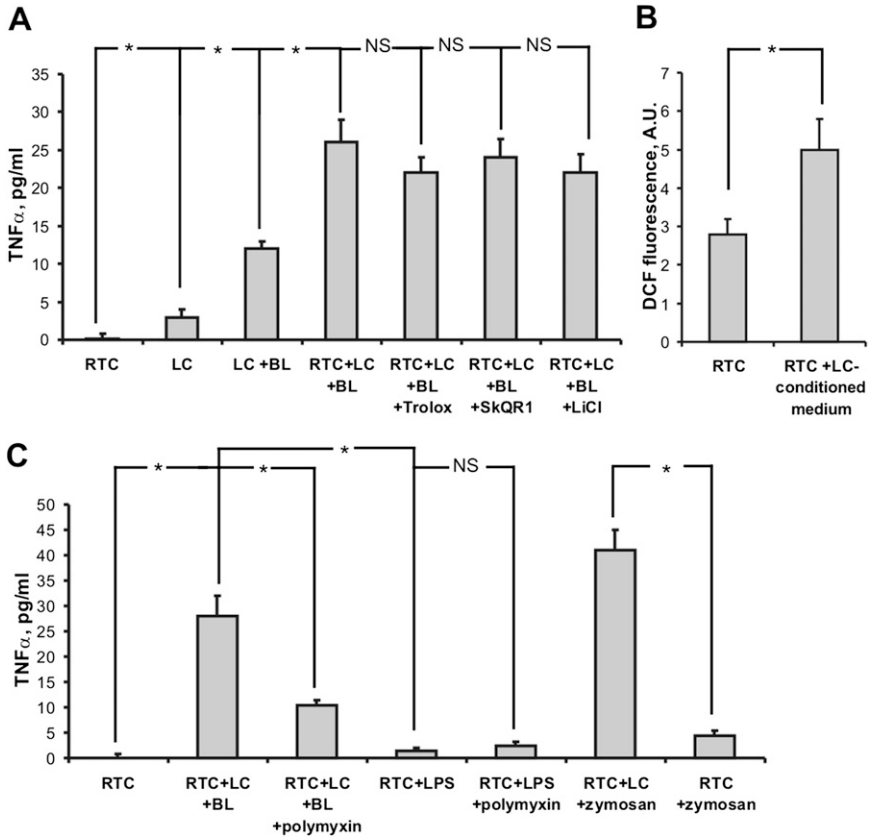

Fig. S4. TNFα production in coculture of renal tubular cells (RTCs) and activated leukocytes (LC + BL). Production of TNFα was significantly higher in activated LCs, compared with control LCs, especially in coculture with RTCs whereas incubation with the indicated drugs had no effect (*A*). In parallel, LC-conditioned medium induced greater DCF fluorescence in renal cells (*B*). (*C*) Effects of different agonists and antagonists of toll-like receptor (TLR) signaling on TNFα production by RTC and LC. *$P < 0.01$.

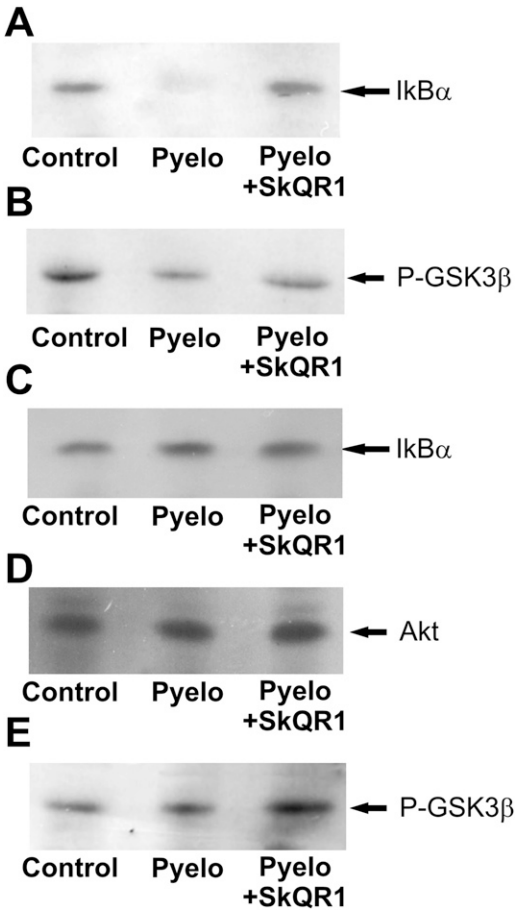

Fig. S5. The state of proinflammatory and antiapoptotic signaling pathways in leukocytes (*A* and *B*) and kidney tissue (*C*–*E*). Leukocytes from pyelonephritic rats demonstrated activation of the NF-κB pathway, as displayed by a decreased level of IκB (*A*) whereas treatment with SkQR1 prevented this activation. Depletion in the P-GSK-3β in leukocytes (*B*) under acute pyelonephritis was partially abolished by SkQR1 pretreatment of the rat. Simultaneously, IκB content (*C*) and concentration of phosphorylated Akt (*D*) were similar for controls and pyelonephritic rats with or without SkQR1 treatment. Levels of phospho-glycogen synthase kinase-3β (P-GSK3β) were similar for controls and pyelonephritic rats but higher in SkQR1-treated rats (*E*).

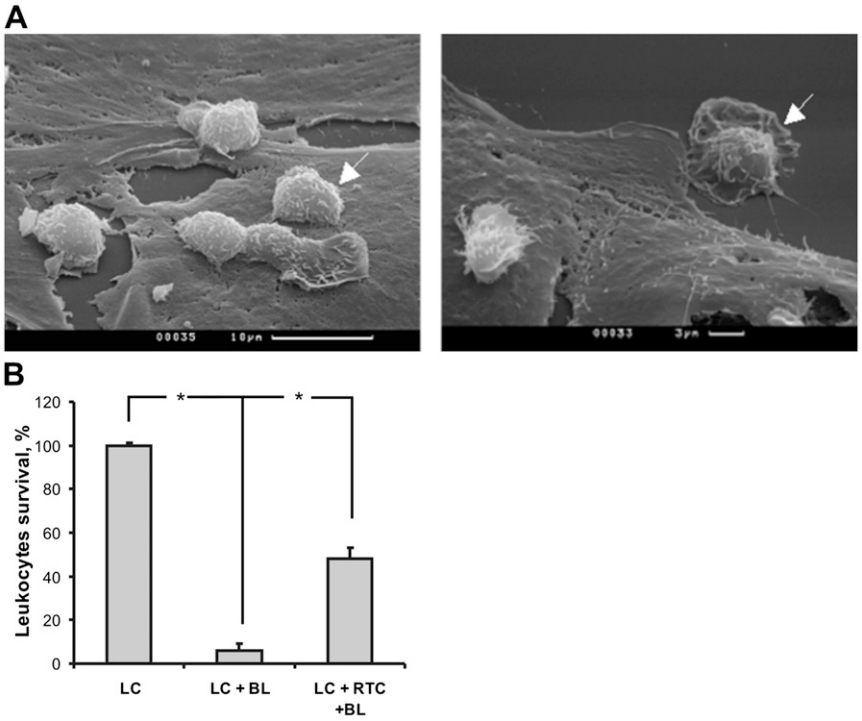

Fig. S6. Intercellular interaction in coculture of renal tubular cells (RTCs) and activated leukocytes (LC). (*A*) Tight-contact communications between leukocytes (arrows) and RTCs after 24 h cocultivation (*Left*). (Scale bar, 10 μm.) Leukocyte attached to a cell-free region of plastic (*Right*). (Scale bar, 3 μm.) (*B*) Leukocyte survival after activation with bacterial lysate (BL) measured by count of GFP-positive leukocytes in the dishes. This interaction was demonstrated in experiments where renal cells were cocultivated with leukocytes carrying GFP, which helped to discriminate between these two kinds of cells. Under cocultivation conditions, the survival of activated leukocytes was higher than that observed without renal cells. *$P < 0.01$.

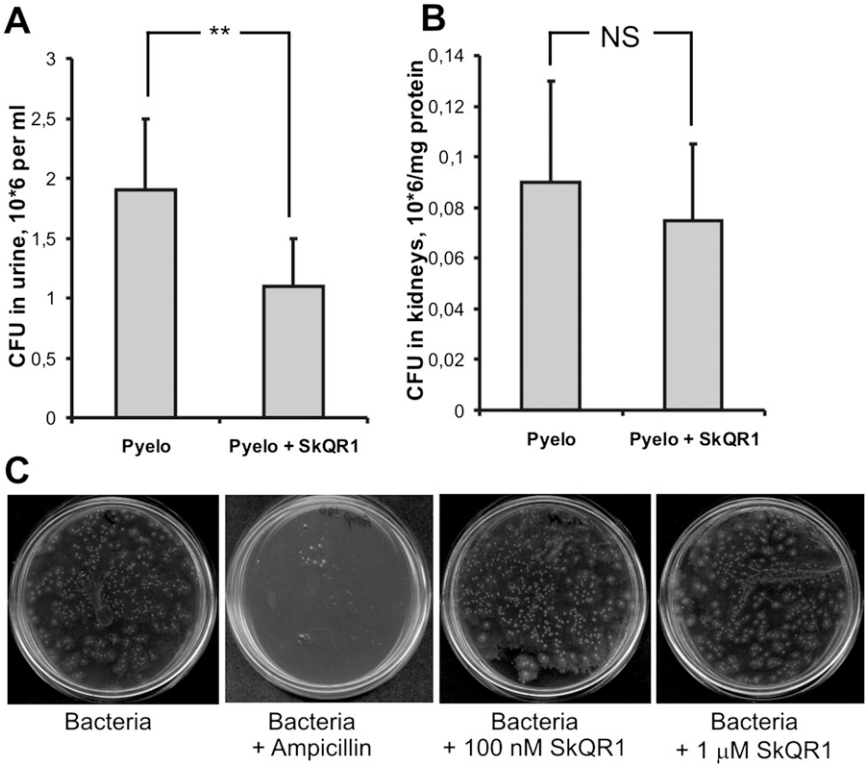

Fig. S7. Effect of SkQR1 on bacterial burden in a rat model of pyelonephritis. In rats treated with antioxidant, the number of cfu in the urine was slightly reduced (*A*) whereas, in the kidneys, it did not change (*B*). SkQR1 didn't demonstrate a bactericidal effect in vitro because even 1 μM SkQR1 in the media did not suppress the growth of bacteria (*C*).