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Cellular and Molecular Life Sciences

Electronic supplementary material

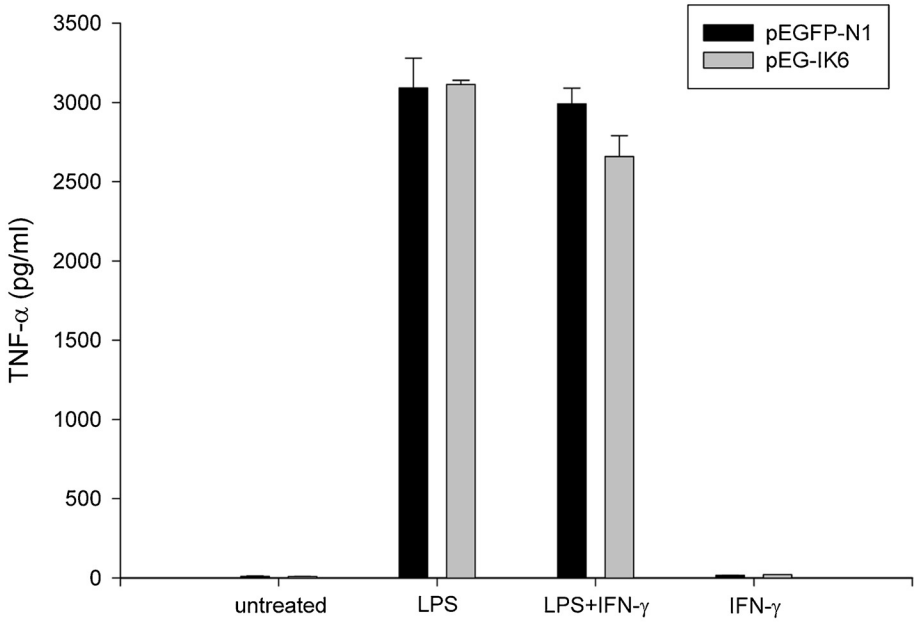
Supplementary Materials and methods

Plasmids. To generate the pCMV-IK6, PCR amplification was performed using the following primers: IK6-F, IK6-XhoI-R (5'-GGGGCTCGAGGCTCAGGTGGTAACGATGCT-3') and pHA-IK1as the template. PCR fragment was digested by *Eco*RI/*Xho*I restriction enzymes and then subcloned into the *Eco*RI/*Xho*I site of pCMV-Taq4A vector (Stratagene, USA).

RT-PCR analysis. The total RNA was isolated from the cultured cells using TRIzol (Invitrogen) according to the manufacturer's instructions. The total RNA was amplified by SuperScript one-step RT-PCR with a PLATINUM Taq kit (Invitrogen). The PCR products were run on a 1.2 % agarose gel and visualized by ethidium bromide staining. The following primers were used to amplify CK2 subunits and GAPDH cDNAs: CK2A1-F (5'-ATGT CGGGACCCGT GCCAAG-3'), CK2A1-R (5'-TTACTGCTGAGCGCCAGCAG-3'), CK2A2-F (5'-ATGCCCGGC CCGGCCGCGGG-3'), CK2A2-R (5'- TCATCGTGCTGCGGTGAGAC-3'), GAPDH-F (5'-CCATGGAGAAGGCTGGGG-3'), GAPDH-R (5'-CAAAGTTGTCATGGATGACC-3'), IK-multi-F (5'-CGATGAGGGTCAAGACATGTCCC-3'), IK-7R (5'-GTCTTCTGCCATCTCGTTGTGG-3'), IK-8R (5'-CATGTCTGACAGGCACTTGT-3')

Immunoblot analysis. The cells were harvested and homogenized by sonication in a homogenization buffer (50 mM Tris-HCl, pH 6.8, 10 % glycerol, 10 % SDS, 0.3 mM PMSF). The protein concentration of the cell lysate was determined using a Bio-Rad protein DC assay (Bio-Rad Lab, Hercules, CA) with BSA (Sigma, St. Louis, MO) as a standard. Cell lysate (20 μg protein) was separated on an 8–15 % SDS-PAGE, transferred to a Hybond ECL membrane (Amersham, Arlington Heights, IL) and probed separately using the anti-iNOS antibody (BD Transduction Laboratories, San Jose, CA) and anti-Ikaros (M-20, Santa Cruz Biotech, Santa Cruz, CA) antibody. The blots were developed using a Westzol plus chemiluminescence kit (Intron). In all immunoblotting experiments, the blots were reprobed with the anti-actin antibody (Sigma) to control the protein loading.

Supplementary Figures



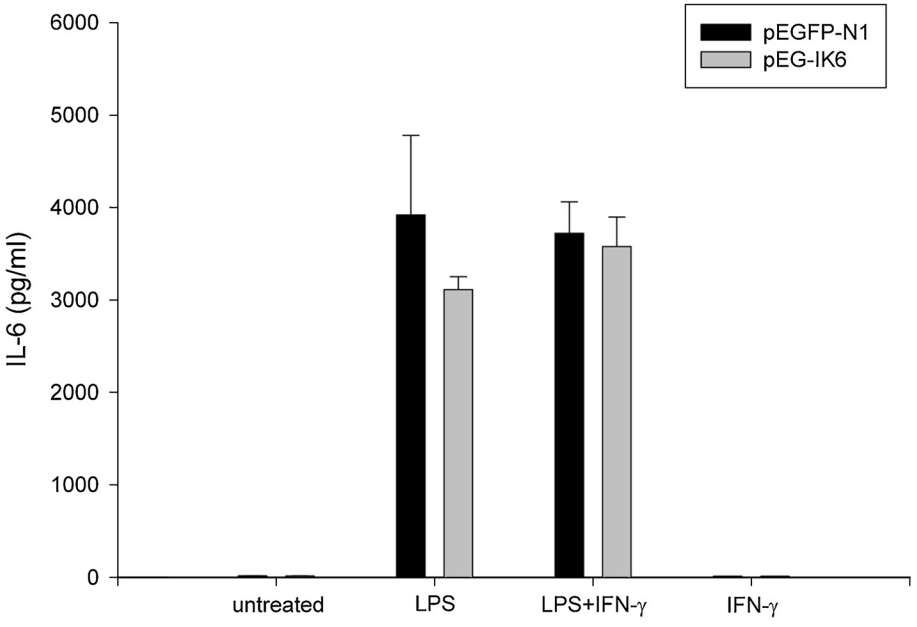


Figure 1. Effect of IK6 overexpression on TNF-α and IL-6 production. IK6-transfected cells were treated with LPS (1 μg/ml) and/or IFN-γ (50 U/ml) for 24 h. The levels of TNF-α and IL-6 were determined using ELISA kit (BD OptEIA) according to manufacturer's recommendation. All the results are representative of at least three independent experiments.

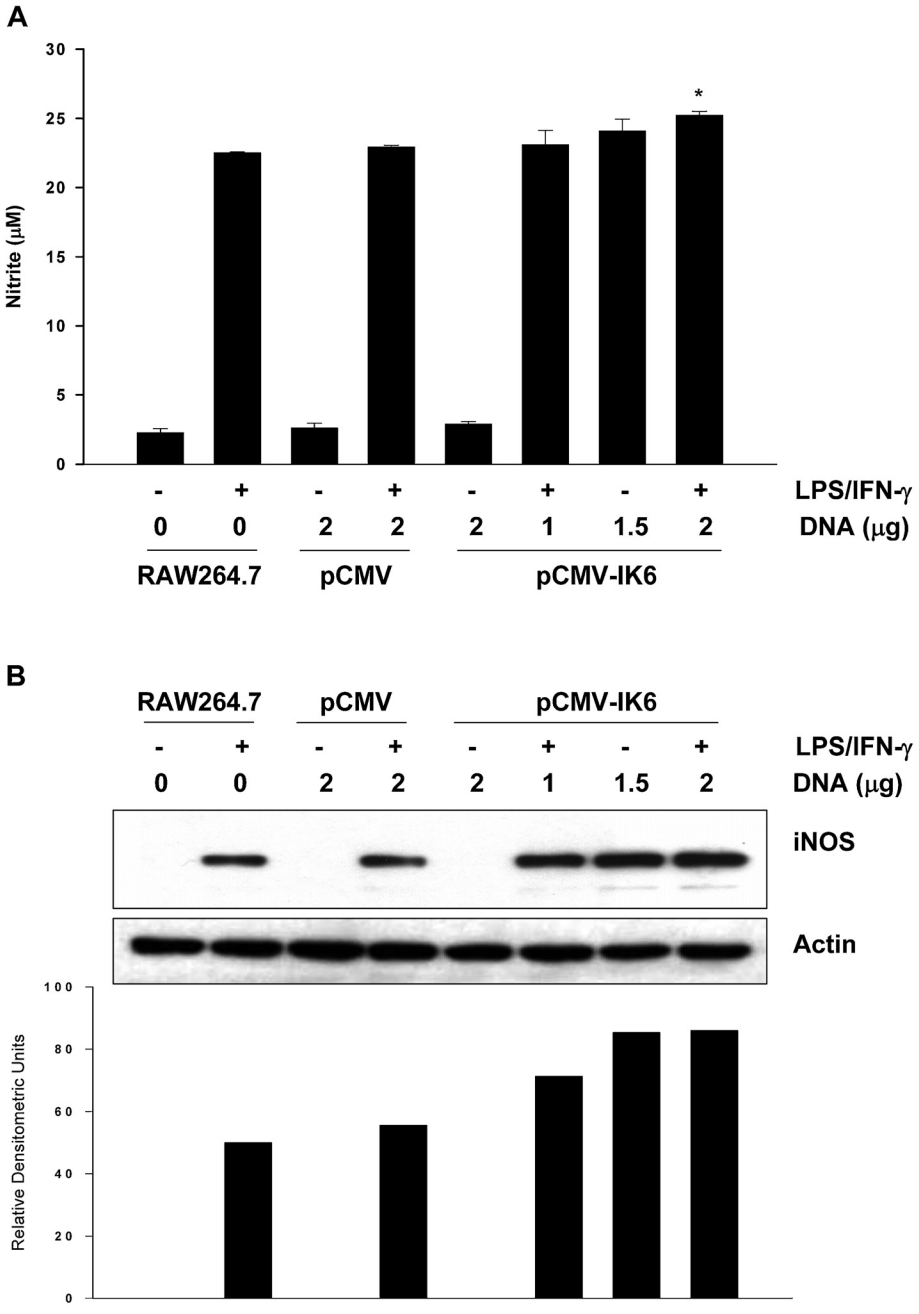


Figure 2. Effect of IK6 overexpression transiently on NO production and iNOS expression. (*A*) RAW264.7 cells were transiently transfected with the vector carrying the IK6 isoform at the indicated doses and were treated with LPS (1 μg/ml) and/or IFN-γ (50 U/ml) for 24 h. NO production was determined using a Griess reagent. (*B*) iNOS expression was determined by an ECL immunoblot assay using the anti-iNOS antibody. The protein level was analyzed by computer-assisted densitometry. A representative experiment is shown that was reproducible at least three times.

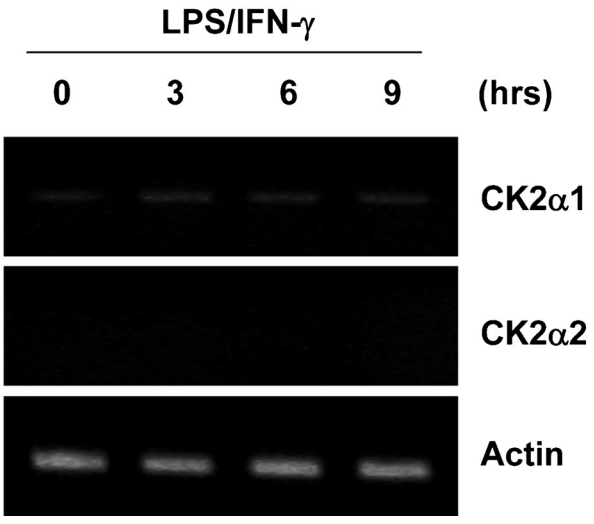
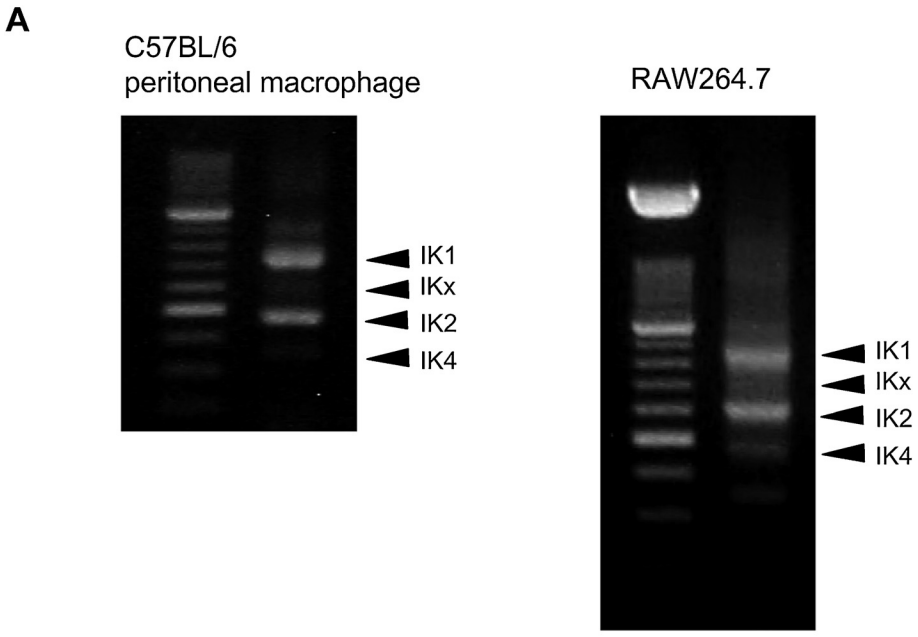
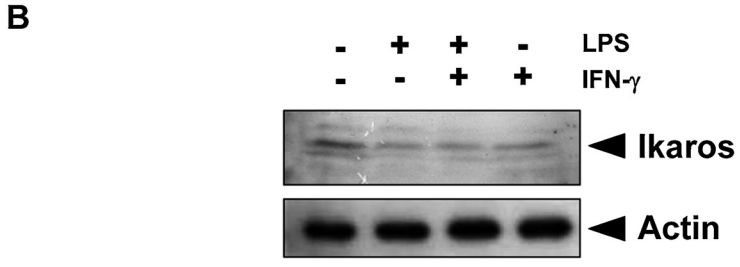


Figure 3. Expression of CK2 subunits in LPS/IFN-γ-activated macrophage. RAW264.7 cells were treated with LPS (1 μg/ml) and/or IFN-γ (50 U/ml) for indicated times. Total RNA was isolated and RT-PCR was performed using primers as described in Methods and materials. GAPDH was served as a normalized control. A representative experiment is shown that was reproducible at least three times.





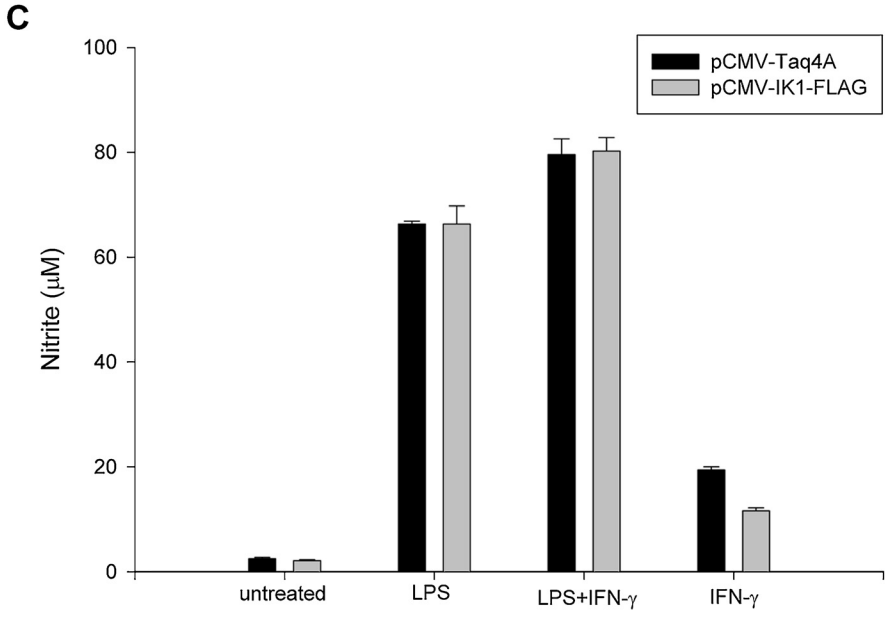


Figure 4. (*A*) To determine the expression patterns of Ikaros isoforms in peritoneal macrophage and RAW264.7 cells, total RNA was isolated and RT-PCR was performed using primers (IK-multi-F, IK-7R, IK-8R) as described in Methods and materials. GAPDH was served as a normalized control. (*B*) Western blot analysis was performed using anti-Ikaros antibody in RAW 264.7 cell after treatment of LPS (1 μg/ml) and/or IFN-γ (50 U/ml) for 24 h. (*C*) To determine the effect of IK1 overexpression transiently on NO production, RAW264.7 cells were transiently transfected with the vector carrying the IK1 isoform at the indicated doses and were treated with LPS (1 μg/ml) and/or IFN-γ (50 U/ml) for 24 h. NO production was determined using a Griess reagent. All the results are representative of at least three independent experiments.