Supplemental Methods

Quantitative real-time PCR (qPCR)

Total RNA was extracted using the TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The concentration and integrity of total RNA was determined using the NanoPhotometer^{TM} spectrophotometer (IMPLEN GmbH, Munich, Germany). Reverse transcription was performed using random hexamer primers and the Superscript II reverse transcriptase kit (Applied Biosystems). qPCR was performed using ABI 7900 sequence detector with TaqMan gene expression assays (Applied Biosystems). ROCK2 mRNA levels were calculated using the difference in threshold cycle method with normalization to the house keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Western blot

Cells were harvested using RIPA buffer (1% Nonidet P-40, 0.5% deoxycholate, and 0.1% SDS in PBS) containing protease inhibitor cocktail (Calbiochem, La Jolla, CA) and were centrifuged at 12,000 rpm for 10 min at 4°C. Protein lysates (20 μg) were denatured in 2% SDS, 10 mM dithiothreitol, 60 mM Tris-HCl (pH 6.8), and 0.1% bromophenol blue. Protein samples were separated on 10% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) and transferred to polyvinylidene difluoride

(PVDF) membranes (Merck-Millipore, Darmstadt, Germany). The membranes were incubated with rabbit anti-ROCK2 (1:1000; abcam, Cambridge, UK) and mouse anti-β-Actin antibodies (1:10000; Santa Cruz, Dallas, Texas) and subsequently treated with the goat anti-rabbit or goat anti-mouse horseradish peroxidase (HRP)-conjugated secondary antibodies (Invitrogen). The blots were visualized by enhanced chemi-luminescence (ECL; Santa Cruz) using the LAS-3000 imaging system (Fujifilm, Japan), and blot intensity was measured by National Institutes of Health Image 1.61.

Human leukocyte isolation

To isolate human leukocytes, 20 ml of peripheral blood sample was mixed with Hanks balanced salt solution (HBSS) in a 50-ml citrate-containing tube. Ten milliliters of Histopaque-1077 (Sigma-Aldrich, St. Louis, MO) was layered and centrifuged at room temperature for 30 mins at 1400–1500 rpm. The supernatant containing the leukocytes was aspirated, mixed with HBSS, and diluted with 2% dextran (1:1 ratio). After centrifugation, red blood cells were lysed and the resulting leukocyte pellet was suspended in medium 199 (Sigma-Aldrich).