Supplementary information

Antibodies Anti-CD44 (clone 156-3C11), anti-Ku70 (clone N3H10 or S5C1), anti-Ku80 (clone 111 or S10B1), anti-CD14 (clone B-A8), anti-transglutaminase (clone CUB 7402 + TG100), monoclonal antibodies were from Neomarkers (Fremont, CA, USA). Anti FGF-2 (clone bFM-2) monoclonal antibody was from Upstate Biotechnology (New York, USA). Anti-cathepsinD monoclonal antibody was from BD biosciences (San Jose, CA, USA). Anti-IL-1β and anti-giantin antibodies were from Abcam (Cambridge, UK). Monoclonal antibody anti-CAK was generously given by Dr. JM Egly (IGMBC, Strasbourg, France).

Cell culture. THP-1 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD) and were grown as described (Monferran et al., 2004). THP-1 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD) and were grown as described (Monferran et al., 2004).

Cell Treatment. When indicated, 5µg/ml of brefeldin A, 20µM of Monensin, 10 µg/mL of cycloheximide, 2 µM of cytochalasin D or 10 µM of nocodazol (all drugs obtained from Sigma, Saint-Quentin-Fallavier, France) was added to the culture medium.

Flow cytometry. Flow cytometry experiments were performed as previously described (Monferran et al., 2004). For PI-PLC treatment, overnight M-CSF treated monocytes were incubated 1hr at 37°C in the presence or not of 1 U / mL of PI-PLC (Sigma, Saint-Quentin-Fallavier, France). Cells were washed with PBS and analyzed by flow cytometry as described. For nocodazol or cytochalasin D treatment, resting monocytes were incubated 2 hr at 37°C with 10 ng/ml of M-CSF in the presence or not of 2 µM of cytochalasin D or 10 µM of nocodazol (Sigma, Saint-Quentin-Fallavier, France). Cells were washed with PBS and analyzed by flow cytometry. For some experiments, stained cells were added to slides pre-coated with L-polylysine, fixed as described (Monferran et al., 2004) and analyzed by confocal microscopy.

Migration assays. Cell migration was evaluated as previously described (Akimov and Belkin, 2001). Briefly, peripheral blood monocytes were labeled with ^{35}S methionine (Trans^{35}S-label, ICN, Orsay, France) overnight in the presence of 10 ng/ml M-CSF. After two washes in serum-free medium, cells were resuspended at 1x10 ^{6} cells/ml. Cells were pre-

incubated for 1hr at room temperature with monoclonal antibodies directed against Ku70 (mAbN3H10), Ku80 (mAbS10B1), blocking antibodies against β1 or β3 or control immune mouse IgG at a final concentration of 20 μg/mL. Then, $1x10^5$ cells in serum-free serum-free IMDM medium supplemented with 10 ng/mL M-CSF were incubated in Costar Transwells (BD Biosciences) with the undersurface coated with 20 μg/mL of fibronectin, or with collagen I, collagen IV, laminin and BSA (10 μg/mL each, all obtained from Sigma). To stimulate chemotactic migration of the cells, 125 ng/mL of monocyte chemoattractant protein 1 (MCP-1) (Sigma) was added to the lower chamber of the Transwells. **In certain experiments increasing concentrations of MCP-1 (from 30 to 125ng/mL) were added to the upper wells.** After 4 hours incubation, non-migrated cells were removed by wiping the upper sides of the membranes with cotton swab. The number of the transmigrated cells present in the undersurface of the inserts, the media and the bottom of the wells were determined by counting the ^{35}S radioactivity in a scintillation counter. The ^{35}S radioactivity was converted into the number of cells by referring to the levels of ^{35}S-radioactivity incorporated per 10^4 cells. At least three independent experiments were performed in duplicate for each condition.

Whole-cell and membrane extraction and Triton X-114 Phase Partitioning. Whole cell extracts were prepared as previously described (Muller et al., 2001). The membrane extracts were prepared as previously described (Yu and Stamenkovic, 1999). Triton X-114 phase partitioning was carried out essentially as previously described (Bordier, 1981). Briefly, crude membranes were resuspended in 1X TBS with 0.25 volumes of Triton X-114 stock solution and extracted at 4°C for 15 min. Insoluble aggregates were then removed by centrifugation and the supernatant was transferred to a new tube. After heat-induced phase transition, the detergent-protein micelles (detergent phase) was collected by centrifugation. The upper aqueous phase was extracted three more times with 0.25 volume of Triton X-114 stock as described above. The detergent phases were pooled together and analyzed by Western Blot

Immunofluorescence. Monocytes, grown on coverslips for the indicated time in presence of 10ng/ml of M-CSF or 1μg/mL of LPS, were fixed in 3.7 % paraformaldehyde for 20 minutes at RT. Immunofluorescence experiments were performed as previously described (Monferran et al., 2004). Confocal images were obtained by means of a confocal laser microscopy system (TCS, NT) (Leica, St-Gallen, Switzerland) fitted with appropriate filter sets. Images were collected by scanning stained cells sequentially under 100X objective lens (zoom 5). For each

sample, over 100 cells were examined in at least three independent experiments. Images were processed for publication using the Adobe PhotoShop 7.0 software program.

References:

Akimov, S.S. and Belkin, A.M. (2001) Cell surface tissue transglutaminase is involved in adhesion and migration of monocytic cells on fibronectin. *Blood*, **98**, 1567-1576.

Bordier, C. (1981) Phase separation of integral membrane proteins in Triton X-114 solution. *J Biol Chem*, **256**, 1604-1607.

Monferran, S., Paupert, J., Dauvillier, S., Salles, B. and Muller, C. (2004) The membrane form of the DNA repair protein Ku interacts at the cell surface with metalloproteinase 9. *Embo J*, **23**, 3758-3768.

Muller, C., Monferran, S., Gamp, A.C., Calsou, P. and Salles, B. (2001) Inhibition of Ku heterodimer DNA end binding activity during granulocytic differentiation of human promyelocytic cell lines. *Oncogene*, **20**, 4373-4382.

Yu, Q. and Stamenkovic, I. (1999) Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. *Genes Dev*, **13**, 35-48.