Miro-1 Links Mitochondria and Microtubule Dynein Motors To Control Lymphocyte Migration and Polarity

Giulia Morlino,a,b Olga Barreiro,a,b Francesc Baixauli,a,b Javier Robles-Valero,c José M. González-Granado,a Ricardo Villa-Bellosta,d Jesús Cuenca,e Carlos O. Sánchez-Sorzano,a Esteban Veiga,fg Noa B. Martín-Cófreces,a,b Francisco Sánchez-Madrida,b

Vascular Biology and Inflammation Department, Centro Nacional Investigaciones Cardiovasculares, Madrid, Spain; Servicio de Inmunología, Instituto Investigación Sanitaria Princesa, Madrid, Spain; Centro de Investigación del Cáncer, CSIC-Universidad de Salamanca, Salamanca, Spain; Department of Epidemiology Atherothrombosis and Imaging, Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain; Unidad de Biocomputación, Centro Nacional de Biotecnología (CSIC), Universidad Autónoma de Madrid, Madrid, Spain; Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología, CSIC, Madrid, Spain; Unidad de Investigación, Hospital Santa Cristina, Madrid, Spain

The recruitment of leukocytes to sites of inflammation is crucial for a functional immune response. In the present work, we explored the role of mitochondria in lymphocyte adhesion, polarity, and migration. We show that during adhesion to the activated endothelium under physiological flow conditions, lymphocyte mitochondria redistribute to the adhesion zone together with the microtubule-organizing center (MTOC) in an integrin-dependent manner. Mitochondrial redistribution and efficient lymphocyte adhesion to the endothelium require the function of Miro-1, an adaptor molecule that couples mitochondria to microtubules. Our data demonstrate that Miro-1 associates with the dynein complex. Moreover, mitochondria accumulate around the MTOC in response to the chemokine CXCL12/SDF-1α; this redistribution is regulated by Miro-1. CXCL12-dependent cell polarization and migration are reduced in Miro-1-silenced cells, due to impaired myosin II activation at the cell uropod and diminished actin polymerization. These data point to a key role of Miro-1 in the control of lymphocyte adhesion and migration through the regulation of mitochondrial redistribution.

The recruitment of blood leukocytes to the site of inflammation involves a sequential, multistep adhesion cascade between the leukocyte and endothelial cell adhesion molecules that mediates leukocyte rolling, firm adhesion, and transmigration across the endothelium (1). Firm arrest of leukocytes on endothelial cells is mediated by the interaction of leukocyte integrins, mainly very late antigen 4 (VLA-4) (α4β1) and lymphocyte function-associated antigen 1 (LFA-1) (αLβ2), with their respective endothelial counterreceptors vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) (2). During this process, the leukocyte and endothelium cytoskeletons undergo an extensive reorganization that ensures sufficient duration of the contact and enables leukocyte extravasation. Leukocyte navigation through tissues is governed by extracellular signals, such as chemoattractant gradients (chemotaxis) and adhesion signals. Chemokines, by activating specific receptors of the G-protein-coupled receptor (GPCR) family, generate leukocyte polarity by inducing the formation of a leading edge (through actin polymerization) and a uropod, a slender posterior appendage characterized by actin-myosin-driven contraction (3, 4).

Mitochondria are highly dynamic organelles that continuously remodel their shape and size through fission and fusion events. They are actively relocalized within the cell through a cytoskeleton-based transportation system (5). These organelles move along microtubules in both anterograde and retrograde directions, using kinesin and dynein motor proteins, respectively (6). Specific subcellular locations with high energy and Ca2+-buffering requirements, such as cellular synapses and other polarized structures, require the establishment of a high local density of mitochondria (7, 8). In leukocytes, mitochondria relocalize to the uropod during chemotaxis (9) and to the immune synapse (IS) to enable modulation of its architecture and downstream signaling (10–12). The molecular mechanisms driving this mitochondrial positioning to specific subcellular locations in lymphocytes are, however, not well understood.

It has been reported that the atypical Rho GTPase Miro-1 plays an essential role in the regulation of mitochondrial morphogenesis and trafficking along microtubules (13, 14) and might serve as a calcium-dependent sensor for the control of mitochondrial motility (15–17). Miro-1 binds the cytoplasmic adaptor protein Milton and kinesin heavy chain through its cytoplasmic domains, thereby connecting mitochondria to microtubules (18). Miro-1 contains a transmembrane domain that anchors it to the outer mitochondrial membrane, two GTPase domains, and two Ca2+-sensing EF-hand domains that protrude into the cytoplasm (14). Upon binding of Ca2+ to its EF-hand domains, Miro-1 dissociates from microtubules (19). The expression of a Miro-1 form with a double mutation in its EF-hands prevents the arrest of mitochondria in response to cytoplasmic Ca2+ elevation (15–17) and decreases the level of Ca2+ entering mitochondria (20).

Here we show that the lymphocyte mitochondria specifically redistribute to the adhesion zone in close contact with the endothelium. Our results indicate that Miro-1, through the regulation of mitochondrial movement along microtubules and its associa-
tion with dynein/dynactin motors, influences mitochondrial positioning. Deficiency in Miro-1 prevents correct interaction with inflamed endothelium, lymphocyte polarization, and chemotactic migration.

(Giulia Morlino conducted this study in partial fulfillment of the requirements for a Ph.D. in Molecular Medicine, Program in Basic and Applied Immunology, San Raffaele University, Milan, Italy.)

MATERIALS AND METHODS

Cells, plasmids, and cell transfection. Human umbilical vein endothelial cells (HUVEC) were obtained and cultured as previously described (21). To activate HUVEC, tumor necrosis factor alpha (TNF-α) (20 ng/ml; R&D Systems) was added to the culture medium 20 h before adhesion/migration assay. Human peripheral blood lymphocytes (PBLs) and T lymphoblasts were obtained and cultured as described elsewhere (22). CEM T cells, CH7C17 T cells, and the human erythrocyte leukemic cell line K562 stably overexpressing puromycin from InvivoGen and hygromycin B from Roche. PureCol (Advanced DiO, DiD, and Dil Vybrant cell labeling solutions, and all fluorochrome-conjugates, JC-1, Fluo4-AM, Mitotracker Orange, Mitotracker Green, fluorescent cell tracker BCECF-AM, Alexa Fluor 488, and 647 phalloidin uptake inhibitor Ru360 and antibiotic G418 were from Calbiochem. The GFP-Miro construct was a gift from J. T. Kittler (University of Padua, Italy). The EB1-GFP construct was a kind gift from Anna Akhmanova (Utrecht University, The Netherlands). The quik change protocol (Stratagene). Small interfering RNAs (siRNAs) against human dynein heavy chain (DHC) were previously described (25); those against anti-pSer19 myosin light chain (MLC) were from Cell Signaling Technologies; monoclonal p50-dynamitin, anti-p150glued and anti-CXCR-4 were from BD Transduction Laboratories; and anti-GFP was from Living Colors (Clontech Laboratories, Inc.). Anti-Miro-1 for Western blotting was from Abcam. Horseradish peroxidase (HRP)-conjugated secondary antibodies were from Pierce. Recombinant human CXCL12 was from Immunotools. Integrin inhibitors BIO5192 and BIRT377 were provided by Biogen Idec and Boehringer Ingelheim, respectively (27).

Fluorescence confocal microscopy. For immunofluorescence assays, lymphocytes were plated on slides coated with PLL or FN or on confluent HUVEC for 20 min at 37°C, fixed with 4% paraformaldehyde (PFA) (Electron Microscopy Sciences), stained with the indicated antibodies, and mounted in Prolong (Invitrogen). Where indicated, the PLL or FN matrix included 10 nM CXCL12. To detect intracellular proteins, cells were permeabilized for 3 min with 0.2% Triton X-100 in phosphate-buffered saline (PBS) before staining. Confocal images were obtained with a Leica TCS-S5 confocal scanning laser unit with an inverted epifluorescence microscope (DMI6000B). Microscopes were maintained at 37°C with 5% CO2 and covered by a full acrylic box, allowing live-cell imaging. For time-lapse fluorescence imaging, cells were plated onto coated 35-mm dishes (MatTek).

Redistribution of lymphocyte mitochondria toward the endothelium was calculated as percentage of the fluorescence intensity (FI) of lymphocyte mitochondria located in the third of the cell near the HUVEC contact area. FI was measured using ImageJ or Metamorph. Cellular polarity was assessed by the elliptical form factor, calculated from images with membrane protein staining using Metamorph software. The phosphorylated myosin light-chain (pMLC) concentration at the uropod with respect to the leading edge and the three-dimensional distance between the mitochondrial network and the microtubule-organizing center (MTOC) were quantified using the Fluorescence Ratio and Distance Measure plug-ins for ImageJ, respectively.

Calcium measurement. For measurement of intracellular Ca2+ flux, control and Miro-1-silenced CEM cells were preincubated with Dil and DiD, respectively, to distinguish them. The pooled cells were loaded with Fluo-4 AM (12) and settled onto MatTek glass-bottom dishes covered by a TNF-α-activated HUVEC monolayer. Images were acquired every 10 s, using a TCS SP5 confocal microscope. The mean Fluoro-4 fluorescence intensity of each migrating cell in each frame was calculated by Wimasis GmbH. Calcium peak intensity was calculated by subtracting the basal fluorescence intensity from the maximum fluorescence registered in each peak. Peak duration was calculated by multiplying the number of frames in which it was registered by frame duration in seconds.

Fluorescence image analysis. Images were analyzed with Leica LASAF (Leica Microsystems), Metamorph (Universal Imaging Corporation, Downingtown, PA), or ImageJ (NIH) software. Mitochondria and MTOC three-dimensional (3D) reconstructions were obtained using Imaris 7.6 software (Bitplane).

Fluorescence ratios. To measure the relative fluorescence between two regions, we computed the ratio between the average fluorescence in the two areas corrected for background fluorescence according to the following model. The model assumes that the observed fluorescence in the different areas can be modeled as the contribution of background fluorescence (Bg), fluorescence in region of interest A (A), and fluorescence in region of interest B (B):
Leica) as described previously (28). For EB1-GFP microtubule plus tip objective and processed with the accompanying confocal software (LCS; 1.5; Mattek, Ashland, MA, USA). Recording was initiated 3 min after cells were allowed to settle onto FN-coated glass-bottom microwell dishes (no. mGFP, were sorted to warrant transfection and cultured for 24 h. Cells (TIRFM), control and Miro-1-silenced CEM cells, transfected with EB1-tracker. EB1-GFP comets were detected by the surface algorithm detecting anomalous nucleation events, tracking of microtubule into movies using Imaris software (Bitplane, Switzerland). Quantification formed through the Leica software. Images were analyzed and converted background fluorescence and that the actual distance between the two regions of interest is isolated in both channels). We then computed the binarized both gray-scale stacks (the user can select two thresholds so that dimensional fluorescence distributions in independent channels, we Distance measurements. To measure the distance between two three-dimensional fluorescence distributions in independent channels, we binarized both gray-scale stacks (the user can select two thresholds so that the region of interest is isolated in both channels). Then we computed the Euclidean distance between every possible pair of voxels (one in distribution A and the other in distribution B). This gives us the exact distribution of pairwise distances. Note that the calculation is fully computed in 3D and therefore that the actual distance between the two regions of interest is calculated. Finally, we computed a truncated mean by computing the distance mean after discarding the 5% lower and upper values. This mean is known to be a robust estimate of the distribution mean.

TIRFM. For total internal reflection fluorescence microscopy (TIRFM), control and Miro-1-silenced CEM cells, transfected with EB1-mGFP, were sorted to warrant transfection and cultured for 24 h. Cells were allowed to settle onto FN-coated glass-bottom microwell dishes (no. 1.5; Mattek, Ashland, MA, USA). Recording was initiated 3 min after cell plating, and cells were visualized with a Leica AM TIRF MC M mounted on a Leica DMI 6000B microscope coupled to an Andor-DU8285, VP-4094 camera. Images were acquired with an HCX PL APO 100.0×1.46 oil objective and processed with the accompanying confocal software (LCS; Leica) as described previously (28). For EB1-GFP microtubule plus tip tracking, penetration was 150 nm for the 488-nm laser channel with same objective angle and a 0.26-s exposure time. Synchronization was performed through the Leica software. Images were analyzed and converted into movies using Imaris software (Bitplane, Switzerland). Quantification of the number of microtubule nucleation events, tracking of microtubule movement trajectories, and computation of microtubule growth were performed with the "connected components" algorithm for particle tracker. EB1-GFP comets were detected by the surface algorithm detection based on channel intensity and using a local background. Analysis was conducted on movie lengths of 300 s that were captured at a rate of 0.5 frame/s. To exclude microtubules that move in and out of the z plane, events shorter than 2 μm were not incorporated into the final analysis.

Ligand-coated microspheres. Sterylene divinybenzene latex beads (6-μm diameter; Sigma) were coated with VCAM-1-Fc or ICAM-1-Fc, produced in our laboratory (29). CH7C17 cells preloaded with Mitotracker Orange (2 × 105) were mixed with precoated latex beads (2 × 105), incubated for 30 min at 37°C in Hanks balanced salt solution (HBSS)–2% bovine serum albumin (BSA), and plated on FN-coated slides. Slides were fixed and immunostained for tubulin. Redistribution of mitochondria and MTOC was considered to have occurred if they were more abundant in the third of the cell centered on the contact with the latex bead.

Immunoblotting and coimmunoprecipitation. After the indicated treatment, CEM and CH7C17 T cells were lysed as described previously (12). For coimmunoprecipitation assays, transfected HEK293T cells or primary T lymphoblasts were lysed in ice-cold lysis buffer containing 0.5% Triton X-100 as described previously (14). Lysates were centrifuged at 16,000 × g for 10 min at 4°C, and the resulting supernatants were preclarified for 1 h at 4°C with protein G Dynabeads (Life Technologies) and incubated for 3 h with anti-GFP or with anti-Miro-1. Immunocomplexes were collected by incubation with protein G Dynabeads for 1 h. The samples were then washed six times with ice-cold lysis buffer and processed for Western blotting. Proteins were separated by SDS-PAGE and visualized with Fujifilm LAS-3000 after membrane incubation with specific primary antibodies and peroxidase-conjugated secondary antibodies. Band intensities were quantified using Image Gauge software (Fujifilm) and results normalized to α-tubulin or, for phosphoproteins, to total protein expression.

Parallel-plate flow chamber analysis of endothelial cell-leukocyte interactions and detachment. PBLs, T lymphoblasts, peripheral blood mononuclear cells (PBMCs), and CEM cells (1 × 106/ml) were drawn across activated confluent HUVEC monolayers at an estimated shear stress of 1.8 dynes (dyn)/cm2 (assumed as physiological for human post-capillary venules where transmigration takes place) for the required time. The shear stress was applied as described previously (23).

Control and Miro-1-silenced CEM cells were first labeled with DiO, DIL, or DiD and then with Mitotracker Orange or Green. Cells (106/ml from each type) were drawn across on activated confluent HUVEC monolayers with a shear stress of 1.8 dyn/cm2. Images were acquired every 30 s. Cells labeled with different cell trackers or Mitotracker displayed similar results, confirming that there was no interference with the cellular processes analyzed.

For detachment experiments, control and Miro-1-silenced CEM cells were preincubated with Mitotracker Orange or Green to distinguish them and allowed to adhere for 5 min at 37°C to activated HUVEC monolayers. Shear stress was started at 0.5 dyn/cm2 and increased up to 15 dyn/cm2. Cell detachment was calculated by normalizing the number of adhered cells to the number observed at the flow rate of 0.5 dyn/cm2.

Adhesion assays. HUVEC were grown to confluence in 96-microwell plates (Costar) and activated with TNF-α. CEM or CH7C17 cells were labeled with 1 μM BCECF-AM and adhered to the activated HUVEC (21). In other experiments, CEM cells (1 × 105/ml) were left untreated or pretreated with 4 μM oligomycin or 10 μM Ru360 (30 min) and washed and resuspended in HBSS–2% FCS. The cells were then adhered to activated HUVEC in 6-well plates for 30 min at 37°C under static conditions or with continuous rotation (60 rpm). After incubation, the plates were washed three times with HBSS, and adhered cells were fixed with 4% PFA. The effects of inhibitors on lymphocyte adhesion were assayed by counting the number of adhered cells using the CellCounter ImageJ plug-in. Results were normalized to those for untreated cells adhered to activated HUVEC. Cells adhered to nonactivated HUVEC were used as a negative control.

Migration and transmigration assays. Migration assays were performed in 5-μm-pore-size Transwell cell culture chambers (Costar) (30). Transmigration through a confluent monolayer of activated HUVEC was assayed in 5-μm-pore-size Transwell plates (23). The number of migrated cells was measured by flow cytometry.

3D migration assay. Migration of CEM T cells through a 3D collagen matrix was assayed according to the manufacturer’s instructions (Ibidi). Control and Miro-1-silenced CEM cells were preincubated with DiD or DiO and images were acquired every 5 min for standard assays (see Fig. 4C) and every 3 min for titration experiments (see Fig. 4D). Images were analyzed using the Manualtracking and Chemotaxis Tool ImageJ plug-ins.

F-actin determination. Levels of polymerized actin were measured as described previously (31). Briefly, cells (1 × 105 per well of a 96-well plate) were incubated with 10 nM CXCL12 at 37°C for the indicated times. Cells were then fixed, permeabilized, and stained with phalloidin-Alexa Fluor 647 (Invitrogen). Fluorescence intensity was determined in a FACSCanto flow cytometer (BD Biosciences) and analyzed with FlowJo software.
Results are expressed as the fold increase in mean fluorescence intensity relative to that of the cells not exposed to CXCL12.

**Flow cytometry calcium measurement.** To track intracellular calcium levels in cells in suspension, control and Miro-1-silenced CEM cells were incubated with DiD or Dil and Fluo-4 AM, and after 1 min of baseline recording, CXCL12 was added (10 nM). Calcium flux was measured over time using a FACS Canto flow cytometer. Ionomycin (0.5 mg/ml) was used as a positive control. Data were analyzed with FlowJo software.

**ATP measurement.** For intracellular ATP quantifications, frozen cells were homogenized with a lysis buffer (25 mM Tris-HCl [pH 7.5], 0.1% Triton X-100), and ATP was rapidly measured by a coupled luciferin-luciferase reaction (32) with an ATP determination kit (Invitrogen, Paisley, United Kingdom). For protein quantification, a colorimetric method (Pierce bicinchoninic acid [BCA] protein assay kit) was used following the manufacturer’s instructions.

**Mitochondrial membrane potential.** The mitochondrion-directed fluorescence-sensitive probe JC-1 was used to determine variations in mitochondrial membrane potential ($\Delta \psi_{m}$) (12). Briefly, CEM T cells (1 × 10^5 per well of a 96-well plate) were preloaded with JC-1 (2 mg/ml) and stimulated with 10 nM CXCL12 at 37°C for the indicated times. FL1 (520 nm) and FL2 (590 nm) emissions were recorded in a FACS Canto flow cytometer and analyzed with FlowJo software.

**Statistical analysis.** Statistical analysis and comparisons were made with GraphPad Prism5. Data were tested for normality using the D’Agostino-Pearson omnibus normality test or using the Kolmogorov-Smirnov test when the sample was small due to experimental conditions. Differences between means were tested by Student’s t test for normal data. Data were analyzed using one-way analysis of variance (ANOVA) with the Bonferroni posttest (for Fig. 2D and 5D and for Fig. S4A in the supplemental material) and with the Tukey posttest (for Fig. 7D) or using two-way ANOVA with the Bonferroni posttest (for Fig. 3D).

**RESULTS**

**Mitochondrial positioning to the adhesion zone during leukocyte-endothelial cell interaction.** We first assessed the specific redistribution of leukocyte mitochondria during the interaction between leukocyte and endothelial cells. Using time-lapse confocal microscopy, we tracked lymphocyte mitochondria, using mitochondrion-targeted fluorescent proteins or with Mitotracker Orange, during their interaction with TNF-α-activated primary human umbilical vein endothelial cells (HUVEC). During static adhesion of human T lymphoblasts to activated HUVEC, most lymphocyte mitochondria localized to the leukocyte-HUVEC contact zone. During subsequent polarization of the migrating lymphoblast, we observed a further relocation of mitochondria toward the uropod (Fig. 1A; see Movie S1 in the supplemental material). The initial redistribution of mitochondria to the contact zone during endothelial adhesion was also observed in the T-cell line CEM under both static and flow conditions (Fig. 1B). This biphasic mitochondrial repositioning, with initial concentration at the endothelial contact zone followed by polarization to the uropod in migrating cells, was also seen under physiological flow conditions and with freshly isolated leukocyte populations (see Fig. S1A and B in the supplemental material). These data indicate that redistribution of mitochondria to the adhesion zone and later to the uropod constitutes a cellular signature of the attachment of leukocytes to endothelium and their subsequent migration.

Mitochondria bind microtubule-associated proteins (MAPs) and move along the microtubule network (6). To monitor the redistribution of the microtubule-organizing center (MTOC) and mitochondria in lymphocytes adhering to activated endothelium (Fig. 1C), we transfected human T lymphoblasts with paxillin-GFP (33) and mRFP to simultaneously track the MTOC and mitochondria (Fig. 1D). During lymphocyte adhesion, most lymphocyte mitochondria and the MTOC moved in a concerted fashion to the contact zone with the endothelium (Fig. 1D). In the reverse experiment, in which endothelial mitochondria were tracked by labeling with mtYFP, we did not detect a significant shift of position upon lymphocyte adhesion (Fig. 1E). Confocal microscopy revealed that endothelial mitochondria colocalized with tubulin but not with the actin cytoskeleton (see Fig. S1C in the supplemental material). In addition, endothelial mitochondria were not present at the docking structure (23) formed after leukocyte arrest on the endothelium (see Fig. S1D in the supplemental material). These results indicate that mitochondrial redistribution is a specific process in leukocytes during interaction with endothelium.

**Mitochondrial redistribution depends on lymphocyte integrin engagement.** We next assessed whether mitochondrial relocation to the contact zone with endothelium is dependent on specific interactions between lymphocyte integrins and adhesion molecules present on activated endothelium. For this, we added lymphoblasts to resting or TNF-α-activated HUVEC and quantified mitochondrial density as the percentage of the total mitochondrial fluorescence intensity located in the contact area (the third of the cell adjacent to the contact site) (Fig. 2A). Upon adhesion to activated endothelial cells, about 60% of mitochondrial fluorescence was present at the contact area, contrasting with 30% upon adhesion to nonactivated endothelium (Fig. 2A). To dissect the role of lymphocyte integrins, we incubated T lymphocytes with beads coated with ICAM-1 or VCAM-1, the ligands of integrins LFA-1 and VLA-4, respectively, or with BSA as a negative control (Fig. 2B). Engagement of VLA-4 or LFA-1 by their specific ligands significantly increased translocation of both mitochondria and MTOC. The translocation rates of both MTOC and mitochondria in response to VLA-4 engagement (with fold inductions in comparison to BSA of 2.1 for MTOC and 1.65 for mitochondria) were higher than those observed with LFA-1 (1.5 and 1.25, respectively) (Fig. 2B and C). Furthermore, T lymphoblasts treated with specific LFA-1 or VLA-4 inhibitors and added to TNF-α-activated HUVEC showed a clear reduction in mitochondrial translocation in comparison to the untreated cells (Fig. 2D), as assessed by mitochondrial fluorescence intensity localized at the contact area with endothelium. These data demonstrate that specific engagement of both VLA-4 and LFA-1 is sufficient to promote MTOC and mitochondrial translocation toward the cell-to-cell contact during lymphocyte adhesion to endothelium.

Retrograde movement of mitochondria along microtubules (toward the minus end, where MTOC is allocated) is mediated by dynein (6). Cytoplasmic dynein is a multisubunit microtubule motor complex that, together with its activator dynactin, drives vesicular cargo toward the minus ends of microtubules (34). Specific silencing of dynein heavy chain (DHC) in T lymphocytes (see Fig. S2A in the supplemental material) uncoupled mitochondria from the MTOC and impaired the recruitment of both organelles to the contact zone (see Fig. S2B in the supplemental material). These cells showed an impaired ability to adhere to activated HUVEC (see Fig. S2C in the supplemental material).

**Miro-1 regulates mitochondrial redistribution and firm lymphocyte adhesion to activated endothelium.** Miro-1 is a calcium-dependent sensor that controls mitochondrial motility along microtubules and therefore mitochondrial trafficking (14-17). We examined the possible involvement of Miro-1 in miot
chondrial translocation during lymphocyte adhesion. Depletion of Miro-1 with a specific siRNA (Fig. 3A), which did not affect Miro-2 expression (see Fig. S3A in the supplemental material), decreased mitochondrial translocation in T cells adhered to activated HUVEC (Fig. 3B), suggesting that Miro-1 controls mitochondrial positioning in response to integrin engagement in T cells. In contrast to DHC-silenced cells (see Fig. S2C), Miro-1-depleted ones showed a slight but nonsignificant decrease of cell adhesion under static conditions (Fig. 3C). A critical step during lymphocyte interaction with the endothelium is firm adhesion. Therefore, we analyzed the role of Miro-1 in firm adhesion by measuring the resistance of T lymphocytes to be detached from endothelium under increasing shear stress. Importantly, Miro-1-silenced T cells detached more readily than controls from HUVEC

FIG 1 Mitochondrial dynamics during leukocyte-endothelium interaction. (A) Representative images from a time-lapse video microscopy analysis of human T lymphoblasts loaded with Mitotracker Orange (red) and adhered to a TNF-α-activated HUVEC monolayer transfected with ICAM-1–GFP (green). Maximal projections of stacks near the endothelial monolayer are shown; dashed lines delineate cell contours of adhering lymphocytes, and arrows mark sites of leukocyte mitochondrial accumulation. Ad, adhered lymphocyte; LE, lymphocyte leading edge; U, lymphocyte uropod. (B) Time-lapse sequence of orthogonal maximal projections of CEM T cells transfected with mtRFP (fluorescence intensity color gradient) adhering to an activated HUVEC monolayer transfected with ICAM-1–YFP (gray scale). The graph shows the percentage of T-cell mitochondrial fluorescence intensity at the contact area (illustrated in the boxed region at time zero, representing a third of the cell close to the HUVEC monolayer; see scheme). Data are shown as a scatter plot; horizontal lines depict medians from two independent experiments of nonpolarized cells adhered to activated HUVEC (**, P < 0.01; ***, P < 0.001 [by one-way ANOVA]).

FIG 2 Mitochondrial redistribution depends on integrin engagement. (A) Quantification of mitochondrial redistribution in lymphoblasts adhered to resting or activated HUVEC monolayers. Mitochondrial redistribution is calculated as the percentage of mitochondrial fluorescence intensity (maximal Z-stack projections of Mitotracker Orange staining) located in the contact area with endothelial cells (defined as the third of the cell close to the HUVEC monolayer; see scheme). Data are shown as a scatter plot; horizontal lines depict medians of 23 and 25 unpolarized cells adhered, respectively, on either TNF-α-activated or nonactivated HUVEC. Three independent experiments were performed (***, P < 0.001). The scheme illustrates the method used to quantify mitochondrial redistribution. (B) Representative images of conjugates formed between CH7C17 cells and latex beads coated with BSA, ICAM-1, or VCAM-1, stained for α-tubulin (green) and mitochondria (red). DIC, differential interference contrast. Scale bars, 10 μm. (C) Fold inductions, in comparison to a BSA negative control, of MTOC and mitochondrial relocalization toward the coated beads. A minimum of 30 (BSA), 95 (VCAM-1), or 140 (ICAM-1) cells were analyzed for each experiment. (D) Quantification of mitochondrial redistribution in lymphoblasts untreated (n = 43) and pretreated (15 min at 37°C) with BIRT377 (n = 41) or BIOS192 (n = 31) to block LFA-1 or VLA-4 integrins and incubated with activated TNF-α-activated HUVEC for 20 min. Data are shown as a scatter plot; horizontal lines depict medians from two independent experiments of nonpolarized cells adhered to activated HUVEC (**, P < 0.01; ***, P < 0.001 [by one-way ANOVA]).
at a flow rate of 5 dyn/cm² or above (Fig. 3D), suggesting a specific role of Miro-1 in regulating the strength of lymphocyte adhesiveness. To explore the role of Miro-1 in lymphocyte polarization, we analyzed the shape of lymphocytes during adhesion to HUVEC under flow conditions by measuring their elliptical form factor. Interestingly, under these conditions, hyperpolarized cells were present in control but not Miro-1-silenced cells (see Fig. S3 in the supplemental material). We next investigated whether the defects observed under physiological flow conditions both in firm adhesion and polarization were accompanied by impaired mitochondrial dynamics. Miro-1-depleted cells presented an evident impaired ability to firmly adhere, polarize, and migrate (see Movie S2 in the supplemental material). Moreover, mitochondrial redistribution to the uropod during polarization and the subsequent migration were greatly affected (Fig. 3E; see Movie S3 in the supplemental material).

Miro-1 knockdown impairs lymphocyte chemotaxis. To assess whether Miro-1 regulates lymphocyte migration capability, we examined CXCL12-induced migration of control and Miro-1-silenced cells in modified Boyden chemotaxis chambers coated with a TNF-α-activated HUVEC monolayer or uncoated. In both cases, Miro-1-silenced cells migrated less efficiently along the chemotactic gradient (Fig. 4A and B). Miro-1 knockdown also disrupted the directionality and reduced the speed of T-cell migration toward CXCL12 in 3D collagen gels (Fig. 4C). These data indicate that expression of Miro-1 is essential for proper directional lymphocyte migration in response to a chemotactic stimulus not only under conditions greatly dependent on integrin function but also in an integrin-independent migration mode (35). We next assessed the contribution of Miro-1 during lymphocyte migration within 3D gels containing different collagen concentrations. Remarkably, Miro-1-silenced cells migrate slower than control cells through different collagen densities (Fig. 4D). It is noteworthy that both control and Miro-1-depleted cells showed the same trend in the decrease of mean velocity at increasing collagen densities (35).

Miro-1 controls CXCL12-mediated lymphocyte polarization and mitochondrion-MTOC association. Chemokines can increase lymphocyte adhesion to endothelium and also induce the acquisition of a polarized, promigratory cell shape (36). CXCL12-induced polarization was impaired in Miro-1-silenced cells (Fig. 5A).

Upon Ca²⁺ binding to its EF-hands, Miro-1 undergoes a conformational change resulting in the dissociation of mitochondria from microtubule track (19). Therefore, we studied polarization after CXCL12 stimulation in lymphocytes overexpressing GFP (as a negative control), GFP-Miro, or GFP-MiroKK, a Miro-1 form with the double mutation E208K E328K that is incapable of Ca²⁺ binding in its EF motifs (14). The overexpression of Miro-1KK with EF motif mutations was able to decrease CXCL12-induced polarization (Fig. 5B; see Movie S4 in the supplemental material). Moreover, we assessed the effect on CXCL12-mediated polarization of Miro-1KK overexpression in cells previously silenced for Miro-1. As shown in Fig. S4A in the supplemental material, the overexpression of GFP-MiroKK did not restore cell polarity whereas GFP-Miro1 rescued the phenotype observed in control cells, underlining the importance of Miro-1 calcium binding domains for its correct function in chemokine-induced lymphocyte polarity.

We next assessed whether the defect in adhesion strength of Miro-1-silenced cells as well as in polarization of both Miro-1-silenced and MiroKK-expressing cells might be related to impaired association of mitochondria with the MTOC. For this, we plated T cells on a fibronectin (FN) or poly-L-lysine (PLL) matrix containing CXCL12 and measured the distance between the center of mass of mitochondrial fluorescence and the MTOC. In control cells, CXCL12 stimulation reduced the distance between mitochondria and the MTOC at the uropod (Fig. 5C and D; see Movie S5 in the supplemental material). This effect of CXCL12 was not observed in Miro-1-silenced cells (Fig. 5C and D; see Fig. S4B and Movie S5 in the supplemental material), supporting the importance of Miro-1 in the relocation of the MTOC and mitochondria to the uropod. Moreover, T cells overexpressing GFP-MiroKK showed a reduced proximity of MTOC and mitochondria induced by CXCL12 in comparison with T cells overexpressing GFP-Miro (Fig. 5E and F; see Fig. S4C and Movie S6 in the supplemental material). Upon chemokine stimulation, mitochondria first redistribute toward the uropod (9) together with the MTOC. Our results indicate that Ca²⁺ binding to Miro-1 EF-hands might lead to a conformational change in Miro-1, resulting in the halt of mitochondria in this specific subcellular location.

The effect of targeting Miro-1 by either silencing or GFP-MiroKK was exerted specifically at the mitochondrion/MTOC polarized association, since other cell polarity parameters such as membrane receptors or cytoskeletal proteins (ICAM-3 or ERM) remained unaltered (see Fig. S4B and C in the supplemental material). To rule out possible pleiotropic effects produced by Miro-1 knockdown on the microtubular skeleton, we have assessed parameters such as the general conformation and dynamics of the tubulin network and the localization of the Golgi apparatus (GA) (37). Neither the tubulin network nor the GA was apparently altered. Hence, the GA showed its usual localization around the MTOC in both control and silenced cells (see Fig. S5A in the supplemental material).
supplemental material). Moreover, the analysis of microtubule dynamics in control and Miro-1-silenced cells showed similar microtubule growth rates (siCtrl, 14.60 ± 3.11 μm·min⁻¹; siMiro, 15.01 ± 2.82 μm·min⁻¹) (see Fig. S5B and Movie S7 in the supplemental material). Furthermore, membrane expression of several cell markers, including integrins and the CXCL12 receptor CXCRI, as well as the CXCL12-mediated endocytosis of CXCR4, was largely unaffected (see Fig. S3C and D in the supplemental material).

These results indicate that Miro-1 mediates mitochondrial positioning and MTOC association, a phenomenon specifically implicated in the chemokine-induced acquisition of a polarized morphology by lymphocytes.

Miro-1 associates with the dynein complex and contributes to regulate CXCL12-induced lymphocyte activation. To investigate how Miro-1 controls mitochondrial positioning, we transfected HEK293T cells with GFP-Miro-1 or GFP alone and examined GFP immunoprecipitates from these cells. The dynein p74 intermediate chain and the dynactin p50 subunit were specifically detected in GFP–Miro-1 immunoprecipitates (Fig. 6A). Therefore, we analyzed this interaction in endogenous complexes from human T lymphoblast lysates. Dynein heavy chain and the p150⁵⁰⁰⁰ component of dynactin were communoprecipitated with Miro-1 in these cells (Fig. 6A, right panel). Hence, Miro-1 appears to be a specific mediator of the interaction between mitochondria and the dynein/dynactin motor complex.

To explore the molecular basis of inhibited polarization in Miro-1-silenced T cells, we studied molecular hallmarks of chemokine-induced lymphocyte polarization. One of these hallmarks is rapid actin polymerization (38), and we detected decreased actin polymerization in response to CXCL12 in Miro-1-silenced cells (Fig. 6B and C). Another polarity hallmark in T cells is myosin light-chain (MLC) phosphorylation, which is required for efficient retraction of the uropod by myosin II (30). Interestingly, CXCL12-induced MLC phosphorylation was inhibited in the uropods of Miro-1-silenced cells (Fig. 6D), suggesting a specific role of Miro-1 in cell polarization.

We next examined the intracellular calcium dynamics in response to CXCL12 stimulation and during lymphocyte migration on activated endothelium. Interestingly, when cells were stimulated with CXCL12 in suspension, we observed no significant differences between control and Miro-1-silenced cells (Fig. 7A). In contrast, when T cells adhered and migrated on activated endothelium, Miro-1 depletion had a negative impact on the intensity and duration of calcium peaks (Fig. 7B and C). These data indicate that Miro-1 regulates intracellular calcium not on a general scale but in a subcellular context.

Mitochondrial activity is reflected in the electrical potential (∆ψm) across the inner membrane and has a direct influence on axonal transport (39). It has been shown that an increased ∆ψm enhances the rate of mitochondrial transport, most obviously retrograde movement (40). We found that CXCL12 increased ∆ψm, but no significant differences were observed between the responses of control and Miro-1-silenced T cells (see Fig. S6 in the supplemental material). Miro-1 depletion thus appears to affect CXCL12-induced movement of mitochondria without significantly affecting their intrinsic activation capacity. Moreover, to rule out possible alterations in mitochondrial functioning due to the downmodulation of Miro-1 expression, we assessed ATP cellular content in both control and Miro-1-silenced cells. Control and Miro-1-silenced cells had similar ATP contents, and CXCL12 stimulation led to a significant reduction in ATP levels in both (Fig. 7D).

Chemokine receptors activate multiple signaling pathways, leading to varied functional outcomes, including adhesion, polarization, and chemotaxis (41). Among these, the ERK1/2 phosphorylation is one of most characterized cell signaling signatures (42). Therefore, we assessed the role of Miro-1 in the modulation of signaling downstream of the CXCL12 receptor through the study of the phosphorylation kinetics of ERK1/2. Maximal ERK1/2 phosphorylation was detected within 2 min of chemokine stimulation in control T cells, and this effect was clearly inhibited in Miro-1-silenced cells (Fig. 7E).

**DISCUSSION**

In this study, we demonstrate that mitochondria relocate to the contact area during lymphocyte adhesion to endothelial monolayers and that this is directed by the GTPase Miro-1. Redistribution of mitochondria is specific to leukocytes and is not observed in endothelial cells. The movement of mitochondria involves the LFA-1/ICAM-1 and VLA-4/VCAM-1 receptor/counterreceptor pairs.

It has been reported that the MTOC and mitochondria can be recruited by chemokine-activated LFA-1 at the immunological synapse (43). Here, we provide evidence that the signal generated by VLA-4 is even more efficient in the redistribution of both MTOC and mitochondria. In our model, mitochondrial arrest in the cellular zone of integrin engagement might be related to local actin polymerization and increased intracellular Ca²⁺. The cytoplasmic tail of the α4 subunit of VLA-4 interacts with paxillin (44), which is a docking protein for other cytoskeletal proteins such as F-actin, for tyrosine kinases, and for GTPase-activating proteins (45). Moreover, VLA-4 engagement can promote the tyrosine phosphorylation of a broad range of proteins, including proline-rich tyrosine kinase 2 (Pyk2), focal adhesion kinase (FAK), and c-Src (46), resulting in the activation of several pathways. Accordingly, increased intracellular Ca²⁺ and LFA-1 engagement triggers activation and redistribution of Pyk2 to the MTOC in T lymphoblasts (47). All these signaling cascades might...
be involved in the confinement of mitochondria and MTOC at the contact zone.

The regulation of the interaction of mitochondria with the cytoskeleton is crucial for proper mitochondrial function at specific sites of action (6). In our search for a specific adaptor between mitochondria and microtubules, we focused on Miro-1, a member of the mitochondrial Rho GTPase family of proteins together with Miro-2. Our observations on Miro-2 expression levels suggest that Miro-1 plays unique functions in mitochondrial localization that cannot be replaced by Miro-2. Miro-1 localizes at the outer mitochondrial membrane and has two EF-hand Ca\(^{2+}\) binding domains (13). Our data show, for the first time, that Miro-1 interacts with the dynein/dynactin complex, whereas interaction of Miro-1 with kinesin was previously described to promote anterograde mitochondrial movement (14, 15, 17, 18). This interaction may account for previously described effects on retrograde mitochondrial movement (48). In this regard, kinesin and dynein are interdependent in axonal transport (49), interacting directly through kinesin light chains (50) and indirectly through the same region of p150\(^{glued}\) dynactin (51). These actions coordinate bidirectional movement of organelles along axon microtubules. Recent evidence suggests that kinesin and dynein can interact with the same mitochondrion, determining its complex mobility patterns in axons (52). Mitochondria can buffer the Ca\(^{2+}\) that enters T lymphocytes via store-operated Ca\(^{2+}\) channels (53). A recent study identified Miro-1 as a Ca\(^{2+}\) sensor involved in the regulation of mitochondrial mobility in neurons and heart cell lines (16). Upon Ca\(^{2+}\) binding to the EF-hand domains, a conformational change in Miro-1 provokes the release of mitochondria from microtubules and their arrest at the subcellular site of calcium influx, where they can contribute to the cell’s response to this second messenger (15–17). Miro-1 function has been studied primarily in

---

**FIG 5** Miro-1 regulates cell polarity and mitochondrial relocalization to MTOC. (A) Left panel, quantification of elliptical form factor in control (1,213 cells) and Miro-1-silenced (1,245 cells) CEM T cells plated on a matrix containing CXCL12. Data are shown as a scatter plot. Horizontal lines depict medians from two independent experiments; ***, P < 0.001.** Right panels, representative cells from each treatment group. Scale bars, 10 μm. (B) Quantification of elliptical form factor in CEM T cells overexpressing GFP (345 cells), GFP–Miro-1 (512 cells), and GFP–MiroKK (431 cells) and plated on a matrix containing CXCL12. Data are shown as a scatter plot. Horizontal lines depict medians from two independent experiments; *, P < 0.05. (C) 3D reconstruction of mitochondria (red) and MTOC (α-tubulin, green) in control and Miro-1-silenced CEM T cells plated on a matrix containing CXCL12. Scale bars, 2 μm. (D) Graph showing the distance between mitochondria and MTOC in cells as in panel C. Data are means ± SEM from two independent experiments; ***, P < 0.001 (by one-way ANOVA). The numbers of cells analyzed for each condition were 73 (siCtrl), 60 (CXCL12 siCtrl), 66 (siMiro), and 64 (CXCL12 siMiro). (E) 3D reconstruction of GFP proteins (green), mitochondria (red), and MTOC (α-tubulin, cyan) in CEM T cells overexpressing GFP, GFP–Miro-1, or GFP–MiroKK and plated on a matrix containing CXCL12. Scale bars, 2 μm. (F) Graph showing the distance between mitochondria and MTOC in cells as in panel D. The numbers of cells analyzed for each condition were 115 (GFP), 103 (GFP–Miro-1), and 113 (GFP–MiroKK). Data are means ± SEM from two independent experiments (***, P < 0.001; **, P < 0.01).

---

**FIG 6** Miro-1 connects mitochondria to the microtubule retrograde transport machinery and regulates CXCL12-induced cytoskeleton activation. (A) Coimmunoprecipitation analysis showing association of Miro-1 with the dynein motor complex. Complexes (IP) were precipitated from cell lysates of transfected HEK293 cells (input) with anti-GFP and probed with anti-p74, anti-p50, and anti-GFP (left panel) or from human primary T lymphoblasts with anti-Miro-1 antibody and probed with anti-dynein heavy chain (DHC), anti-p150, and anti-Miro-1 (right panel). (B) Actin polymerization dynamics in control or Miro-1-silenced CEM T cells exposed to CXCL12. F-actin content was measured by flow cytometry and is expressed relative to time zero. Data are presented as means ± SEM from three independent experiments; ***, P < 0.001 (by multifactorial ANOVA). (C) Representative images of pMLC distribution in control and Miro-1-silenced CEM T cells plated on a matrix containing CXCL12. Cells were stained for pMLC (green), Mitotracker Orange (red), and ICAM-3 to mark the uropod (cyan) in maximum projections of a confocal Z-stack of CEM T cells plated on a matrix containing CXCL12. Scale bars, 2 μm. (D) Graph showing the distance between mitochondria and MTOC in cells as in panel C. Data are means ± SEM from two independent experiments; *, P < 0.05. (E) 3D reconstruction of mitochondria (Mitotracker Orange, red), and ICAM-3 to mark the uropod (cyan) in maximum projections of a confocal Z-stack of CEM T cells plated on a matrix containing CXCL12. F-actin content was measured by flow cytometry and is expressed relative to time zero. Data are presented as a scatter plot. Horizontal lines depict medians from two independent experiments; **, P < 0.01.

---

**April 2014 Volume 34 Number 8 mcb.asm.org**

1423
neuronal cells, where soma and synaptic structures can be distant from each other. It is therefore conceivable that mitochondrial positioning influences synaptic transmission by local ATP production and local Ca\(^{2+}\) buffering. Although changes of mitochondrial positioning within small spherical cell like lymphocytes may be more modest (54), the evidence on how mitochondrial morphology and positioning are crucial for cellular signal threshold in immune cells is emerging (9–12, 43, 55). Our data demonstrate how changes of only few micrometers in lymphocyte mitochondrial positioning to the adhesion zone or to the uropod are able to significantly affect both adhesion and migration. Unlike lymphocytes, migrating epithelial cells require an anterior mitochondrial localization for correct migration. In these cells, Miro-1 knockdown also perturbs mitochondrial localization and reduces their migratory capability (56), in agreement with our data. The localized Ca\(^{2+}\) oscillation during migration, and inhibition of ERK1/2 phosphorylation. It is therefore conceivable that Miro-1 regulates through Ca\(^{2+}\) oscillation during migration, and inhibition of ERK1/2 phosphorylation at the rear of the migrating cell. The localized Ca\(^{2+}\) influx may contribute, through calmodulin-based machinery, to the activation of the MLC kinase required for uropod retraction during migration (62, 63). At the same time, mitochondrial Ca\(^{2+}\) influx can increase ATP production by activating the tricarboxylic acid cycle and enhancing the activities of the electron transport chain enzymes and the ATP production of control and Miro-1-silenced CEM T cells (prestained with DiD and DiL, respectively). Results from a representative experiment out of three performed are shown. (B) Representative intracellular calcium oscillation in control and Miro-1-silenced CEM T cells migrating on TNF-α-activated HUVEC. (C) Distribution of relative calcium peak intensity (left) and peak duration in seconds (right). Data are shown as a scatter plot; horizontal lines depict medians from two independent experiments (67 control and 62 Miro-1-silenced cells) (***, P < 0.001). (D) Amounts of ATP in unstimulated and CXCL12-stimulated (10 min) control and Miro-1-interfered cells. Data are means ± SEM from two independent experiments. ***, P < 0.001; *, P < 0.05 (by one-way ANOVA). (E) Time course of the phosphorylation status of ERK1/2 in control and Miro-1-silenced CEM T cells activated with CXCL12. Total protein expression is shown as a loading control. The right panel shows pERK/total ERK band intensity ratios relative to the ratio in control cells at r = 0. Data are presented as the means ± SD from two independent experiments.
synthase complex (64). Whether the impaired mitochondrial re-
distribution to the uropod caused by Miro-1 downregulation is
able to reduce Ca$^{2+}$ entry at this subcellular location remains to be
elucidated. Nevertheless, we cannot rule out that Miro-1 might
play additional roles unrelated to mitochondrial positioning that
might contribute to its effects on actin polymerization, calcium
oscillations, and cell migration.

In summary, we demonstrate that Miro-1 can contribute to the
regulation of mitochondrial translocation toward the MTOC dur-
ing lymphocyte adhesion to the inflamed endothelium and the
subsequent directed migration toward the site of inflammation,
demonstrating the importance of mitochondrial subcellular loc-
alization in these processes. Considering the importance of an ap-
propriate and rapid recruitment of leukocytes for an efficient
immunologic response, our results shed new light about the regulation of
adhesion and migration processes that could help the design of
new, more specific strategies for the treatment of a large variety of
infectious diseases in which there is deregulated leukocyte recruit-
ment.

ACKNOWLEDGMENTS
We thank S. Bartlett for English editing, Joseph T. Kittler for providing
reagents, M. Vicente-Manzanares for critical reading of the manuscript,
and V. Rocha-Pergini for helpful discussions. We thank the CNIC Mili-
croscopy and Celomics units.

This study was supported by SAF2011-25834 from the Spanish
Ministry of Science and Innovation, INDISNET-S2011/BMD-2332 from the Comunidad de Madrid, Red Cardiovascular RD 12-0042-0056 from Instituto Salud Carlos III (ISCIII), and ERC-2011-AdG 294340-
GENTRIS. J.M.G.-G. received salary support from the Miguel Servet
Chair of the Instituto Salud Carlos III (ISCIII), and ERC-2011-AdG 294340-
from the Comunidad de Madrid, Red Cardiovascular RD 12-0042-0056
from the Spanish Ministry of Economy and Competitiveness (JCI-2011-09663).

REFERENCES
4. Vicente-Manzanares M, Sanchez-Madrid F. 2004. Role of the cytoske-
//dx.doi.org/10.1038/nri1268.
5. Detmer SA, Chan DC. 2007. Functions and dysfunctions of mitochondrial
1038/nrm2275.
7. Li Z, Okamoto K, Hayashi Y, Sheng M. 2004. The importance of
dendritic mitochondrial morphology in the morphogenesis and plasticity of spines and
EG, Hoth M. 2007. T cell activation requires mitochondrial translocation to the
12. Baixauli F, Martin-Cofreces NB, Morlino G, Carrasco YR, Calabia-
Linares C, Veiga E, Serrador JM, Sanchez-Madrid F. 2011. The mito-
chondrial fission factor dynamin-related protein 1 modules T-cell re-
doi.org/10.1038/emboj.2011.25.
roles in mitochondrial homeostasis and apoptosis. J. Biol. Chem. 278:
Miro-1 and Miro-2 have essential roles in mitochondrial trafficking.
1016/j.bbrc.2006.03.163.
15. Macaskill AF, Reinholz JE, Twelvetrees AE, Arancibia-Carcamo IL,
Muir J, Fransson A, Aspenstrom P, Atwell D, Kittler JT. 2009. Miro1 is a calcium sensor for glutamate receptor-dependent localization of mito-
16. Cai Q, Sheng ZH. 2009. Moving or stopping mitochondria: Miro as a
1016/j.neuron.2009.09.003.
17. Chang KT, Niescer RF, Min KT. 2011. Mitochondrial matrix Ca2+ as
1106862108.
19. Cereghetti GM, Stangherlin A, Martins de Brito O, Chang CR, Black-
stone C, Bernardi P, Scorrano L. 2008. Dephosphorylation by calcineu-
20. Martin-Cofreces NB, Robles-Valero J, Cabrero JR, Mittelbrunn M,
Gordon-Alonso M, Sun CH, Alarcon B, Vazquez J, Sanchez-Madrid F. 2008. MTOC translocation modulates IS formation and controls sus-
jcb.200800114.
21. Campanero MR, del Pozo MA, Arroyo AG, Sanchez-Mateos P, Hernan-
de-Caselles T, Craig A, Pilulo P, Sanchez-Madrid F. 1993. ICAM-3 inter-
acts with LFA-1 and regulates the LFA-1/ICAM-1 cell adhesion pathway.
PN, Gratton E, Caiolfa VR, Sanchez-Madrid F. 2008. Endothelial adhe-

Downloaded from http://mcb.asm.org on May 1, 2016 by guest


