Role of the nuclear membrane protein Emerin in front-rear polarity of the nucleus

Paulina Nastały, Divya Purushothaman, Stefano Marchesi, Alessandro Poli, Tobias Lendenmann, Gururaj Rao Kidiyoor, Galina V. Beznoussenko, Stefania Lavore, Orso Maria Romano, Dimos Poulikakos, Marco Cosentino Lagomarsino, Alexander A. Mironov, Aldo Ferrari & Paolo Maiuri

Cell polarity refers to the intrinsic asymmetry of cells, including the orientation of the cytoskeleton. It affects cell shape and structure as well as the distribution of proteins and organelles. In migratory cells, front-rear polarity is essential and dictates movement direction. While the link between the cytoskeleton and nucleus is well-studied, we aim to investigate if front-rear polarity can be transmitted to the nucleus. We show that the knock-down of emerin, an integral protein of the nuclear envelope, abolishes preferential localization of several nuclear proteins. We propose that the frontally biased localization of the endoplasmic reticulum, through which emerin reaches the nuclear envelope, is sufficient to generate its observed bias. In primary emerin-deficient myoblasts, its expression partially rescues the polarity of the nucleus. Our results demonstrate that front-rear cell polarity is transmitted to the nucleus and that emerin is an important determinant of nuclear polarity.

1 FIRC (Fondazione Italiana per la Ricerca sul Cancro) Institute of Molecular Oncology (IFOM), Milan 20139, Italy. 2 Laboratory of Thermodynamics in Emerging Technologies, Department of Mechanical and Process Engineering, ETH Zurich, Sonneggstrasse 3, CH-8092 Zurich, Switzerland. 3 Department of Physics, University of Milan, via Celoria, 16 20133 Milan, Italy. 4 EMPA, Swiss Federal Laboratories for Materials Science and Technology, 8600 Dübendorf, Switzerland. ✉ email: paolo.maiuri@ifom.eu
Cell polarity is defined as an intrinsic asymmetry observed in the structural orientation of the cytoskeleton, mainly due to actin filaments and microtubules. It is manifested in cell shape and structure as well as distribution of proteins and cellular organelles. Cellular polarity is crucial in many biological processes, such as morphogenesis, differentiation, proliferation, and migration. Particularly, migration is a fundamentally polarized process that requires the organization of the cell machinery along a front-to-rear axis. Various migratory cell types display a characteristic morphology with a protruding front, at the opposite of a retracting trailing edge. This so-called, front-rear polarity is essential and dictates the direction of movement. It also parallels the polarization of intracellular compartments as well as signaling cascades. However, it is still unknown if and how front-rear polarity of a cell is transmitted to the largest cellular organelle, the nucleus. Seminal studies reported a tension-induced basal-to-apical polarization of lamin A/C in mouse embryonic fibroblasts. However, it has not been further investigated how much this impacts other nuclear envelope (NE) proteins or the nuclear interior.

As it has been previously shown that the nucleus exhibits radial organization, in this study, we aim to analyze it along the front-to-rear axis. To that end, we systematically and quantitatively study spatial distribution of various components in front-to-rear polarized cells. Our findings reveal that the asymmetric organization of the cell can be transmitted to the nucleus.

**Results**

**Distribution map preparation.** To evaluate a possible transmission of polarity from cytoskeleton to nucleus we needed a cell population with a clear front-rear polarity in which to test this hypothesis. We therefore plated human hTERT RPE-1 (RPE1) cells on fibronectin-coated micro-patterned lines 10 μm in width. Cells spreading on such substrates acquire an elongated shape, develop a spontaneous front-rear polarity and randomly migrate in 1D. This condition is considered to partially recapitulate the in vivo environment by mimicking the directional orientation of extracellular matrix fibres that occur within tissue. Afterward, we developed a method to quantitatively analyze spatial protein distribution by combining images of multiple cells spread on the lines. Essentially, images were oriented by the Golgi apparatus, here used as a marker of directionality, filtered, registered for the positioning of the nucleus, and analyzed (Fig. 1b–g). As expected, the Golgi apparatus orientation determined the corresponding positioning of the microtubule-organizing center (MTOC), both towards the putative direction of motion (Fig. 2a, b). This bias was clearly lost in a randomly oriented map (Supplementary Fig. 1a, b) proving the validity of our method. To understand whether polarity could be transmitted from the cytoskeleton to the nucleus, we started to systematically explore the preferential distribution of proteins at the interface between these two cellular compartments: the nuclear envelope (NE).

**Emerin as a candidate involved in polarity transmission.** Emerin (EMD), an integral membrane protein of the nuclear envelope, has been reported to be present at both, the inner (INM) and the outer nuclear membrane (ONM), hence potentially enabling polarity transmission from outside the nucleus to the inside. It was shown to play diverse roles, including chromatin tethering, cellular polarity organization, cell signaling, gene expression, and mechanotransduction. Interestingly, as first evidence of nuclear polarity, we observed a significant frontal enrichment of EMD in front-rear polarized cells (Fig. 2c; Supplementary Fig. 2a, b) Because its peculiar localization at both sides of NE could be determinant for nuclear polarity transmission, we decided to systematically compare protein map distributions in control and EMD knock-down cells.

**Cytoplasmic effects of EMD knock-down.** We first observed that the effects of EMD knock-down (Supplementary Fig. 2i) were not restricted to the nucleus. Indeed, it induced a general rearrangement of the cytoskeleton: the nucleus was positioned peripherally within the cell, and the cells had much shorter retractable tails than the control cells (Fig. 2a, b). Both F-actin and focal adhesion distributions were strongly perturbed (Supplementary Fig. 2j, k). Additionally, it induced a general elongation of the nucleus (Supplementary Fig. 2l). We therefore plated human hTERT RPE-1 (RPE1) cells on fibronectin-coated micro-patterned lines 10 μm in width. Cells spreading on such substrates acquire an elongated shape, develop a spontaneous front-rear polarity and randomly migrate in 1D. This condition is considered to partially recapitulate the in vivo environment by mimicking the directional orientation of extracellular matrix fibres that occur within tissue. Afterward, we developed a method to quantitatively analyze spatial protein distribution by combining images of multiple cells spread on the lines. Essentially, images were oriented by the Golgi apparatus, here used as a marker of directionality, filtered, registered for the positioning of the nucleus, and analyzed (Fig. 1b–g). As expected, the Golgi apparatus orientation determined the corresponding positioning of the microtubule-organizing center (MTOC), both towards the putative direction of motion (Fig. 2a, b). This bias was clearly lost in a randomly oriented map (Supplementary Fig. 1a, b) proving the validity of our method. To understand whether polarity could be transmitted from the cytoskeleton to the nucleus, we started to systematically explore the preferential distribution of proteins at the interface between these two cellular compartments: the nuclear envelope (NE).

**Fig. 1 Distribution map preparation scheme.** a Representative cells migrating on micro-patterned lines. b Random images of single cells. c Orientation of single cells using Golgi signal as a marker of directionality. d Nuclei registration. e Merged image of multiple cells from the dataset. f Color-coded distribution maps of the nucleus, Golgi and actin. g Normalized density plot of the front-to-rear distribution.
**Fig. 2 Mapping the front-rear polarity in the nucleus.** To facilitate the comparison, the distribution maps of control and EMD knock-down cells are presented next to each other. **a** Single control and EMD knock-down RPE-1 cells on 10 μm micro-patterned lines. **b** Distribution maps of the nucleus, Golgi, MTOC, and ER in control (left panel) and EMD knock-down (right panel) cells. The magenta outline represents the F-actin border of the averaged cells. Normalized frequency color-coding used to prepare all the maps in the study. **c** Distribution map of EMD, P = 6 × 10^{-12}, two-sided Kolmogorov-Smirnov test (e) Distribution maps of LEM domain-containing proteins in control (left, P_{Cramer}\text{–von Mises} = 9.1 \times 10^{-11}, P_{MAN1} = 0.010 two-sided Kolmogorov-Smirnov) and EMD knock-down (right, P_{Cramer}\text{–von Mises} = 0.0004) two-sided Cramer-von Mises test) cells. **f** Distribution maps of LINC-complex proteins in control (left, P_{SUN2} = 8.6 \times 10^{-6}, P_{Nesprin-1} = 1.9 \times 10^{-9}, P_{Nesprin2} = 0.005 two-sided Kolmogorov-Smirnov test) and EMD knock-down (right, P_{SUN2} = 2.6 \times 10^{-6}, P_{Nesprin-1} = 0.026, two-sided Cramer-von Mises test) cells. **g** Distribution maps of lamins (LMN), LMN A/C Ser22-Ser22 phosphorylated lamin A/C, in control (left, P_{LMNB1} = 1.4 \times 10^{-10}, P_{LMNCAC} = 4.4 \times 10^{-16}, two-sided Kolmogorov-Smirnov test) and EMD knock-down (right, P_{LMNCAC} = 1.4 \times 10^{-10} two-sided Cramer-von Mises test) cells. **h** Distribution maps of transcription-related markers, nActin—nuclear actin, RNAPII Ser5-Ser5 phosphorylated RNA polymerase II, in control (left, P_{nActin} = 3.8 \times 10^{-8}, P_{H3K4me3} = 0.011, P_{H3K9ac} = 0.030, P_{H3K27me3} = 0.00025, two-sided Kolmogorov-Smirnov test) and EMD knock-down (right, P_{nActin} = 3.9 \times 10^{-6}, P_{RNAPolSer5} = 0.042, P_{H3K4me3} = 2.9 \times 10^{-11}, P_{H3K9ac} = 0.015, P_{H3K27me3} = 0.00023, two-sided Cramer-von Mises test) cells. **i** Distribution map of chromosome 3 in control (left, P = 0.007 two-sided Kolmogorov-Smirnov test) and EMD knock-down (right, P = 0.013 two-sided Cramer-von Mises test) cells. **j** Graphical representation of the DamID technique (left) that serves to mark emerin-associated domains (EADs). Distribution maps of Emerin-Dam and Dam protein alone, P = 7.5 \times 10^{-5}, two-sided Kolmogorov-Smirnov test. ***P < 0.001, **P < 0.01, *P < 0.05, ns—not significant. For each distribution map an exact number of cells from three independent experiments is stated in the figure. Source data are provided as Source Data file.
Fig. 2j). Moreover, the distribution of the Golgi apparatus and MTOC appeared more spread along the cell body, suggesting a systemic relaxation of intra-cellular connections (Fig. 2b). In addition, we noted, as previously reported, a ~2.5-fold increase in nucleus-MTOC distance (Fig. 1d).

**Distribution map of LEM domain-containing proteins.** EMD is one of the LEM domain-containing proteins that are involved in chromatin tethering and gene expression regulation. Interestingly, other members of the family showed different behaviors. MAN1 (also known as LEM domain-containing protein 3), like EMD, has a transmembrane domain (TM), and is localized at NE. As EMD, in polarized cells, it was enriched towards the cell front, if with a milder bias (Fig. 2e; Supplementary Fig. 2a, c). LAP2α (Lamina-associated polypeptide 2α), in contrast, is lacking TM and is localized in the nucleoplasm. In polarized cells it was enriched towards the cell rear (Fig. 2e; Supplementary Fig. 2a, c). The EMD knock-down had diverse effects as it has changed the distribution of LAP2α, without affecting that of MAN1 (Fig. 2e; Supplementary Fig. 2a, c). Hence, one of the LEM domain-containing proteins that are involved in chromatin tethering and gene expression regulation, but there are other members of the family that showed different behaviors.

**Distribution map of nuclear envelope proteins.** The NE is one of the LEM domain-containing proteins that are involved in chromatin tethering and gene expression regulation, and it is also known for its actin-capping properties. Therefore, we decided to investigate the distribution of nuclear actin (nActin). Indeed, nActin was more frequently distributed at the front tip of the nucleus (similar to EMD), and upon EMD knock-down, its distribution shifted to both tips (Fig. 2h; Supplementary Fig. 2a, f). As nActin was mainly linked to transcription, interacting with all types of RNA polymerases, we investigated the distribution of active RNA polymerase II (Ser5P) (Fig. 2h; Supplementary Fig. 2a, f). Interestingly, nActin was more frequently found at the rear tip (Fig. 2h; Supplementary Fig. 2a, f). In control cells, it was equally present at both tips of the nucleus, whereas in EMD-deficient cells, it was more frequently found at the rear tip. In control cells (Fig. 2h; Supplementary Fig. 2a, f). It was slightly different from active chromatin markers. Whereas, H3K4me3 was enriched towards the front in control cells and changed its distribution to the rear upon EMD knock-down (Fig. 2h; Supplementary Fig. 2a, f). H3K9ac was uniformly distributed in the control cells and was shifted to the front in EMD-deficient cells (Fig. 2h; Supplementary Fig. 2a, f). EMD was also shown to be involved in chromatin remodeling by the replacement of H3K9me2,3 with H3K27me3 upon mechanical stress. Therefore, we investigated markers of constitutive (H3K9me3) and facultative heterochromatin (H3K27me3) (Fig. 2h; Supplementary Fig. 2a, f). H3K9me3 showed slight frontal enrichment but occurred predominantly at the sides of the nucleus; its positioning was not affected by EMD knock-down. Instead, H3K27me3 was mainly distributed at the rear, which was even more pronounced in EMD-deficient cells (Fig. 2h; Supplementary Fig. 2a, f). Overlapping distributions of RNAIPII (marker of active transcription) and H3K27me3 (an epigenetic mark of facultative heterochromatin) might seem counterintuitive, however, our data does not imply co-localization of these proteins. The rear (or the front) of the nucleus should be considered as a huge ‘macro-domain’, where both regions of heterochromatin and euchromatin could be enclosed. The spatial resolution of our maps, indeed, which should be considered similar to probability distributions, is not sufficient to infer or exclude the co-localization of different chromatin domains. Moreover, the understanding of the specific molecular mechanisms driving the preferential localization, and re-localization upon EMD knock-down, of the different nucleoplasmic components, clearly warrants follow up study.

**Distribution map of chromosome territories.** Afterward, to understand whether cell polarity could also affect genome spatial organization, we studied chromosome localization. Indeed, chromosomes adopt a conserved and non-random arrangement in sub-nuclear domains called chromosome territories (CTs), and moreover, it has been recently shown that their positioning is partially modulated by EMD. In our analysis, chromosomes 12, 18, and 22 were non-uniformly distributed (Supplementary Fig. 3a–c). Chromosome 3, as the only CT, lost its frontal enrichment upon EMD knock-down (Fig. 2i; Supplementary Fig. 2a, g).

**Emerin-associated genomic domains follow the distribution of EMD.** To further elucidate the functional implications of nuclear polarity, we employed an EMD-DamID system. This technology allows the specific tagging of genomic regions that are in molecular contact with EMD. We demonstrated that emerin-associated genomic domains (EADs) were more frequently found...
at the front side of the nucleus, confirming that the observed protein bias is also transmitted to the chromatin interacting with it (Fig. 2j; Supplementary Fig. 2a, h).

Distribution maps in non-patterned cells. To test that our results were not induced by forcing cells to move on one-dimensional lines we investigated protein distribution in cells plated on fibronectin-coated glass (Supplementary Fig. 4a). As polarity is an intrinsic property of cells and cells plated on 2D surface would still spontaneously establish a polarity axis and move in one direction, we applied our method also in this condition. While the cellular and nuclear shape differed from the micro-patterned ones (Supplementary Fig. 4a, b), however after orienting the cells, the maps for preferential protein positions in non-patterned cells were similar to the micro-patterned ones (Supplementary Fig. 4c–g).

Effect of LAP2α, MAN1, and LMN B1 knock-down also affect nuclear polarity. Because we found that EMD is necessary for the correct polarity of some components of the nuclear envelope we tested if reciprocally the absence of other proteins could affect its distribution. When we knocked-down other nuclear envelope proteins including LAP2α and LMN B1, we observed no effect on EMD preferential distribution (Fig. 3a, c; Supplementary Fig. 5a, b). In cells with MAN1–deficiency, EMD frontal enrichment was even more pronounced (Fig. 3b; Supplementary Fig. 5a, b). On the other hand, nesprin-1 distribution was altered in both MAN1 and LAP2α knock-down, however, its frontal localization remained (Fig. 3a, b; Supplementary Fig. 5b). In LMN B1–knock down condition, nesprin-1 completely lost its frontal enrichment (Fig. 3c; Supplementary Fig. 5b). These results confirm the central role of EMD in nuclear polarity transmission, indeed, EMD is required for the correct localization of other LEM domain-containing proteins, as LAP2α and MAN1, but is not affected but their knock-down. Still, the reduction of both of them impacts on nesprin-1 distribution, as well as the LMN B1 knock-down. These data suggest, first, a possible hierarchy of LEM domain-containing proteins in nuclear polarization and, then, that an integral nuclear envelope is necessary for the establishment of a complete and correct nuclear polarity.

Migrating cells show EMD enrichment. We also analyzed RPE1 cells stably transfected with EMD-EGFP, migrating on micro-patterned lines. Importantly, the EMD bias we observed by combining images from multiple fixed cells could also be observed in living cells (Fig. 4a; Supplementary Movie 1).

EMD plays a role in migration and force transmission. EMD knock-down significantly perturbed cell migratory properties: cells lacking EMD exhibited increased velocity and persistence (Supplementary Fig. 6a) and impaired chemotaxis efficiency (Supplementary Fig. 6b). We also noted that cells with EMD deficiency had smaller focal adhesions (Supplementary Fig. 6c), and accordingly, they transmitted less force to their substrate39 (Fig. 4b; Supplementary Fig. 6d), which could explain the observed increase in speed40,41. Although our results differ from a previous work examining nesprin-1 and lamin A-deficient cells42, they indicate that EMD has a crucial role in force transmission and raise further questions about the cytoplasmic role of EMD and the mechanism that could generate its frontal bias at the NE.

EMD and nesprin-1 localize in the cytoplasm. Immunofluorescent staining for EMD showed that some fraction of the protein localized in the cytoplasm, as was also evident in cells transfected with EMD-EGFP (Fig. 4a). We, therefore, performed transmission electron microscopy (TEM) with immuno-gold labeling to determine the exact localization of EMD. It revealed that EMD is not only present at the INM and ONM, as previously reported, but is additionally found in the cytoplasm on the ER membranes (Fig. 4c). This result is coherent with previous findings reporting initial integration of EMD in the ER membrane, whence it then moves to the ONM, thanks to the continuity between these two compartments, and finally reaches the INM41,43. Additionally, the EMD interactor nesprin-1, an actin-binding protein, was present not only at the ONM and in the nuleoplasm but also in the cytoplasm, where it co-localized with actin filaments (Fig. 4c).

EMD and nesprin-1 bind together in the cytoplasm. To test whether EMD and nesprin-1 can also interact in the cytoplasm, probably linking the ER to the cytoskeleton, we performed a proximity ligation assay (PLA) between these two proteins (Fig. 4d). As a control, we performed a PLA between lamin B1 and EMD. While the PLA foci between lamin B1 and EMD were mostly restricted to the NE, the ones between nesprin-1 and EMD were observed in the NE and extending into the leading.

---

**Fig. 3 Effect of LAP2α, MAN1, and LMN B1 knock-down on nuclear polarity.** a Distribution maps of the nucleus, Golgi, EMD and nesprin-1 in cells with LAP2α knock-down. P_{nesprin-1} = 0.0007, wo-sided Cramer-von Mises test. The magenta outline represents the F-actin border of the averaged cells. b Distribution maps of the nucleus, Golgi, EMD and nesprin-1 in cells with MAN1 knock-down. P_{EMD} = 6.6 × 10^{-5}, P_{nesprin-1} = 0.011, two-sided Cramer-von Mises test. The magenta outline represents the F-actin border of the averaged cells. c Distribution maps of the nucleus, Golgi, EMD and nesprin-1 in cells with LMN B1 knock-down. P_{nesprin-1} = 1.96 × 10^{-7}, wo-sided Cramer-von Mises test. The magenta outline represents the F-actin border of the averaged cells. \(*P < 0.001, **P < 0.01, \*P < 0.05, \) ns—Not significant. For each distribution map an exact number of cells from three independent experiments is stated in the figure. Source data are provided as Source Data file.
cytoplasmic region (Fig. 4d). Moreover, the preferential distribution maps of protein interactions corresponded to the separate maps of each protein (Fig. 4d).

Expression of dominant negative KASH domain displaces EMD from NE. By expressing dominant negative KASH-EGFP (DN-KASH-EGFP) domains that compete with nesprins binding to the SUN proteins, thereby disrupting the LINC complex, we found that in addition to nesprin-1, EMD was also displaced from the nuclear envelope. It was indeed present mainly in the frontal cytoplasm of the cell similarly to DN-KASH-EGFP and nesprin-1 (Fig. 4e; Supplementary Fig. 6g). We showed that perturbing the connection between the cytoskeleton and the nucleus, alters nuclear polarity.

Nuclear polarity persists upon drug treatment. In order to test whether nuclear polarity can be altered by affecting cytoskeleton components, we incubated micro-patterned cells with different agents including cytochalasin D (prevents actin polymerization), blebbistatin (inhibits myosin), calyculin A (enhances myosin), nocodazole (prevents microtubule polymerization). Although all drug treatments clearly affected both cytoskeleton components, nuclear polarity was not altered.
and ER, as shown by the altered cell shape and KDEL distribution map, the ER and Golgi frontal localization was always preserved (Fig. 4f; Supplementary Fig. 6e). In agreement with the literature, we performed drug treatments using sub-lethal drug concentrations for a short time. These conditions are sufficient to perturb the cytoskeleton but not to destroy the polarity of the cell, and indeed, we detected alterations of nuclear polarity, not its complete loss. Specifically, we observed, that although none of the drugs abolished the EMD frontal enrichment, cytochalasin D and nocodazole significantly decreased it (Fig. 4f; Supplementary Fig. 6f). On the other hand, calyculin A slightly increased the EMD frontal polarization (Fig. 4f; Supplementary Fig. 6f), suggesting that an enhancement in contractility, probably generally increasing cell polarity, also induce an increase of nuclear polarity.

A mathematical model supports the hypothesis that front-rear bias in the ER could transmit EMD polarity. Overall, these results suggest that EMD enrichment at the frontal NE originates from the asymmetric distribution of the ER at the leading edge, from which EMD moves to the ONM and further to the INM (Fig. 4g, Supplementary Fig. 6h). To support this view, we developed a minimal mathematical model (Supplementary Information) in which the total amount of EMD entering the ER is assumed to be proportional to the ER surface, and the EMD diffusion in the NE is assumed to be slow enough to be neglected. This simple model reproduces the observed bias of EMD at the NE as a function of the asymmetric distribution of the cytoplasm, Lf — ER frontal length, Lr — ER rear length, LTotal — total ER length. *P < 0.001, **P < 0.01, *P < 0.05, ns — not significant. For each distribution map an exact number of cells from three independent experiments is stated in the figure. Source data are provided as Source Data file.

Discussion

Polarity is an intrinsic property of the cells. Almost all adherent cell types, indeed, independently of external cues, are able to self-define an axis of polarity and migrate. This asymmetry implies an asymmetric organization of the cellular architecture, with a non-uniform distribution along the cytoskeleton of proteins, organelles, and tensile stress.13 While the connection between the cytoskeleton and the nucleus is well-studied, it is unknown if part of the front-rear cell polarity is somehow transmitted to the nucleus.

This study demonstrates that front-rear cell polarity is transmitted from the cytoskeleton to the nuclear envelope and, thus, defines a nuclear polarity. We show that nuclear polarity not only concerns the preferential distribution of proteins at the NE, but also extends to nucleoplasmic proteins and, to some extent, to chromatin. Moreover, we show that EMD, an integral protein of the nuclear envelope, suggested to be involved in mechanotransduction,20,51 is one of the molecular players involved in nuclear polarity transmission. The knock-down of EMD clearly affects the biased distribution along the polarity axis of some components of the nuclear envelope and also in the nucleoplasm. However, our results suggest that there are other molecular components responsible for front-rear polarity of the nucleus, with a complex hierarchy of interactions. For example, the polarity of some elements of the NE, such as nesprin-1 or MAN1, is not affected by EMD reduction. Additionally, also the knock-down of LAP2α, MAN1 or LMN B1 affects the distribution of other NE components, but without perturbing the biased distribution of EMD. This suggests that probably an integral nuclear envelope is necessary for the correct and complete transmission of nuclear polarity.

Notably, we found that protein distribution maps in RPE1 and primary myoblasts, while being similar, did not perfectly match. Therefore, we can conclude that the extent and feature of nuclear polarity are cell-type specific. It is possible that different LINC complex proteins are not only differentially expressed but also distributed in a different manner among various cell types, having either redundant or essential functions.27

We hypothesize that nuclear polarity is generated by the asymmetric distribution of the ER and that it relies on the continuity between the ER and the outer nuclear membrane. Our results also suggest a possible role of the ER in mechno-sensing. Indeed, EMD and nesprin-1, interacting at the surface of the ER,
could generate a continuous meshwork linking the cytoskeleton, the ER and the nucleus, enabling tension transmission along those various compartments.

The pathological consequence of emerin loss is the Emery Dreifuss muscular dystrophy,[53] While we cannot directly prove that the disease is caused by the loss of nuclear polarity, we show that in primary myoblasts from an EDMD patient nuclear polarity is perturbed. With emerin ectopic expression, we were able to partially rescue the normal phenotype. Mutations in other matrix proteins[23] were fabricated using photolithography[13]. The glass surface of the coverslip was activated with plasma cleaner (Harrick Plasma) and then coated with cell repellent (NanoFlair, Cell Sciences). Then, the coverslips were incubated with primary antibodies (as listed in Supplementary Table S1) and proper secondary antibodies (Jackson ImmunoResearch). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich Cat. D8417) and left for 16 h to attach on micro-patterned lines.

**Methods**

**Cell culture.** Human hTERT-immortalized RPE-1 cell line was cultured in DMEM/HAM’S F12 (Bio West, Cat. L0093-500) supplemented with 10% FBS (Euroclone, Cat. ECS0181L). Cells were split every 2–3 days and passaged not more than 6 times. Human primary myoblasts were obtained from Telethon Biobank. Individuals gave written informed consent before undergoing muscle biopsy, in agreement with the Declaration of Helsinki and approved by the ethical committees of the centers, where biological samples were obtained. Normal muscle biopsy (male, catalog#70515) and the one from patient with EDMD (male, catalog#49031, Emd mutation cDNA.539_543delTCTAC) were cultured in DMEM (Lonza, Cat. BE12-614F) supplemented with 20% Fetal Bovine Serum South America (Sigma-Aldrich, Cat. F9665), 10 μg/ml human recombinant insulin (Sigma-Aldrich, Cat. 11376497001), 25 ng/ml human recombinant fibroblast growth factor (Peprotech, Cat. 100-18B), and 10 ng/ml active human recombinant epithelial growth factor (Vinci-Biochem, Cat. BPS-90201-3). Primary cells were split every 3–4 days and for analysis were taken cells at passage 4–10.

**Micro-patterning.** Micro-patterns of fibronectin-coated lines (10 μm of width) were fabricated using photolithography[13]. The glass surface of the coverslip was activated with plasma cleaner (Harrick Plasma) and then coated with cell repellent PPL-g-PEG (Surface Solutions GmbH, 0.5 mg/ml in 10 mM HEPES). After washing with 1x phosphate-buffered saline (PBS) and deionized water, the surface was illuminated with deep UV light (UVO Cleaner, Jelight) through a chromium matrix protein fibronectin (Sigma-Aldrich, Cat. F1056, 25 μg/ml in 100 mM NaHCO3 pH 8.4). Cells were detached with EDTA 0.02% (Versane, Gibco, Cat. E6758) and left for 16 h to attach on micro-patterned lines.

**Immunofluorescence.** Cells on micro-patterns were fixed with 4%PFA/1x PBS, permeabilized in 0.1% Triton-X100 PBS, and incubated in blocking solution (1%BSA in 1x PBS). Then, cells were incubated with primary antibodies (as listed in Supplementary Table S1) and proper secondary antibodies (Jackson Immunoresearch). Nuclei were stained with 4’,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich Cat. D8417) Cells were mounted with Vectashield® Antifade Mounting Medium (Vector Laboratories, Cat. H-1000).

**Chromosome painting.** Fluorescent in situ hybridization was performed using protocol enabling 3D nuclear structure preservation[54]. Briefly, cells were fixed with 4% PFA for 10 min. and immuno-stained with antibody to visualize Golgi apparatus. After post-fixing with 4% PFA for 10 min, the specimens were incubated for at least 60 min. in 20%glycerol/1x PBS, followed by freeze-thawing cycles in liquid nitrogen. The cells were permeabilized in 0.07% Triton-X100/0.1 M HCl for 10 min. and DNA was denaturated in 30% Formamide/25SSC (pH = 7.4) for 10 min. Then, chromosome painting probes (Metsystems, Xyting Chromosome Paints) were added to the specimen, denaturated for 3 min. at 75°C, and

---

Fig. 5 Nuclear front-rear polarity rescue in primary myoblasts. a Single primary normal (left), EDMD (middle) and EMD-EGFP-rescue (right) myoblasts on 10 μm micro-patterned lines. b Distribution maps of the nucleus, Golgi, and MTOC in normal (left panel), EDMD (middle panel) and EMD-EGFP-rescue cells (right panel). The outline represents the F-actin border of the averaged cells. c Measurement of nuclear-MTOC distance (\(n_{\text{normal}} = 123, n_{\text{EDMD}} = 110, n_{\text{EDMD+EGFP}} = 189\) cells from three independent experiments) \(P_{\text{normal vs. EDMD}} = 1.3 \times 10^{-5}, P_{\text{EDMD vs. EDMD+EGFP}} = 0.0013\). Measurement of size of focal adhesion \(n_{\text{normal}} = 103, n_{\text{EDMD}} = 87, n_{\text{EDMD+EGFP}} = 88\) cells from three independent experiments) \(P_{\text{value normal vs. EDMD}} = 1.9 \times 10^{-14}, P_{\text{normal vs. EDMD+EGFP}} = 0.00019, P_{\text{EDMD vs. EDMD+EGFP}} = 0.00071\). The boxes represent the mean values and the line in the box represents median. Whiskers represent the minimum and maximum values. Kruskal-Wallis test. d Distribution map of EMD in primary normal (left, \(P_{\text{normal}} = 3.0 \times 10^{-13}\), two-sided Kolmogorov-Smirnov test) and EMD-EGFP (right, \(P_{\text{EDMD+EGFP}} = 3.4 \times 10^{-5}\), two-sided Cramer-von Mises test). e Distribution map of nesprin-1 in primary normal (left, \(P = 0.004\), two-sided Kolmogorov-Smirnov test), EDMD (middle, \(P = 2.4 \times 10^{-6}\), two-sided Cramer-von Mises test) and EMD-EGFP-rescue (right, \(P = 0.04\), two-sided Cramer-von Mises test) myoblasts. *** \(P < 0.001\), ** \(P < 0.01\), * \(P < 0.05\), ns—not significant. For each distribution map an exact number of cells from 3 independent experiments is stated in the figure. Source data are provided as Source Data file.
hybridized at least 16 h at 37 °C in hybridization chamber. Afterward, the cells were washed for 10 min. in 2×SSC and 0.1%SC buffers. Nuclear were stained with DAPI (Sigma-Aldrich Cat. D8417) and the samples were mounted in Vectashield® Antifade Mounting Medium (Vector Laboratories, Cat. H-1000-10).

**DamD experiment.** This method was previously used to map genome and nuclear lamina contacts37,38. Briefly, 293T cells were transfected with DamID plasmids by calcium phosphate protocol (10 μg DNA for each 10-cm dish). Forty-eight hours later, viral supernatant was collected, 0.45-μm filtered and added to RPE1 cells. For the DamID experiment hTERT RPE-1 cells were co-transfected with either pHIV-TetPuro-ires-EFS-EGFP-DPN7 or pHIV-TetPuro-IRES-EGFP-DPN7 and pl-GW-V5-EcoDam plasmids (control) or pHIV-TetPuro-IRES-EGFP-DPN7 and pl-GW-EcoDam-V5-HEM (visualization of eremin-associated domains)37. The plasmids were a kind gift of Prof. Bas van Steensel (Division of Gene Regulation, Netherlands Cancer Institute, Amsterdam, Netherlands). After 48 h incubation with ribavirin, nuclease was changed and the cells were micro-patterned and left for 16 h to attach. The coverslips containing cells were fixed with 4% PFA and immunostained in order to visualize Golgi and/or emerin.

**Transfection and RNAi.** Cells were transfected with Lipofectamine 2000 reagent (Life Technologies, Cat. 13778030) and 10 nM human gene specific siRNA duplexes (abx903005, Abbrex Ltd) with following sequences:

- **EMD:** 5‘-CtGArArGrArGrArGrArGrArGrUrArUrArCtTA-3’ (MAN1: LEMD3): 5‘-CtGUrGrUrGrUrArUrArUrArUrGrUrGrGrArGrUrAcGtA-3’
- **LAP2a (TMPo):** 5‘-GrArArGrArGcArGrArAcGArAcGrArUrArAaG-3’

RNAi to target MAN1 and LAP2a was performed using Lipofectamine RNAiMAX reagent (Life Technologies, Cat. 13778030) and 10 nM Human gene specific 27mer siRNA duplexes (abx930035, Abbrex Ltd) with following sequence:

LMNB1: 5‘-CgGAcAcUaaCgGcCaaAttTuUggAcGgUuGAcGcTt-3’

As a control, a Trilencer-27 Universal Scrambled Negative Control siRNA Duplex (Origene) was used. Cells were visualized after 48 h post-transfection.

**Stable EMD-EGFP RPE1 cell line generation.** RPE1 cells were transfected with Emerin-pEGFP-C1 plasmid. Forty-eight hours post-transfection cells were cultured in medium containing 10% FBS (Euroclone, Cat. ECS0181) and the samples were fixed in 4% PFA and immunostained in order to visualize the combination of rotation and translation of the source to fit the target. The same roto-translation was applied to all channels. For each channel, all the registered images were combined in a single stack. Then, the stack was segmented using threshold value for each image in the stack using TopHat (value 20) and for pericentrin signal using Otsu (value 100) for nuclearus and Otsu (value 100) for nucleus. Finally, the custom-built ImageJ macro measured distance between the centrosome and the closest border of the nucleus for each cell. Value of 0 means that centrosome was positioned above the nucleus.

**Time-lapse microscopy for migration analysis.** RPE1 cells were plated on micro-patterned lines 16 h before imaging. In order to track the cells, nuclei were stained with NucBlue live stain (Thermo Fisher Scientific, Cat. R37605). Cells were imaged using a confocal microscope. For each channel all the registered images were combined in a single stack. Then, the stack was segmented using threshold value for each image in the stack, using Yen (value 25) for pericentrin signal and Otsu (value 100) for nucleus. Finally, the custom-built ImageJ macro measured distance between the centrosome and the closest border of the nucleus for each cell. Value of 0 means that centrosome was positioned above the nucleus.

**Centrosome-nucleus distance analysis and quantification.** Immuno-fluorescent staining using anti-pericentrin antibody was performed and nuclei were visualized using DAPI. After projecting Z-stacks, nuclei were registered to the reference nucleus using the ImageJ plugin. The combination of rotation and translation of the source to fit the target was segmented using threshold value for each image in the stack using TopHat (value 20) and for pericentrin signal using Otsu (value 100) for nucleus. Finally, the custom-built ImageJ macro measured distance between the centrosome and the closest border of the nucleus for each cell. Value of 0 means that centrosome was positioned above the nucleus.

**Focal adhesion analysis and quantification.** In order to visualize focal adhesions, cells were stained with anti-paxillin antibody. Using the custom-built ImageJ macro, the best z-plane was selected, the signal was segmented using Intermodes.
Transmission electron microscopy. Electron microscopic examination and immune EM gold-labeling based on pre-embedding, were performed as previously described, a detailed description is explained below. EM based Morphological analysis: RPE1 cells grown on MatTek glass-bottom dishes (MatTek Corporation, Cat. P35G-1.5-C-14) for 16h. Samples were fixed with 4% paraformaldehyde and 2.5% glutaraldehyde (EMS) mixture in 0.2 M sodium cacodylate pH 7.2 for 2 h at RT, followed by 6 washes in 0.2 sodium cacodylate pH 7.2 at RT. Then cells were incubated in 1:1 mixture of 2% osmium tetroxide and 3% potassium ferrocyanide for 1 h at RT followed by 6 times rinsing in cacodylate buffer. The sections were sequentially treated with 0.3% Thiocarbohydrazide in 0.2 M cacodylate buffer for 10 min and 1% OsO₄ in 0.2 M cacodylate buffer (pH 6.9) for 30 min. Then, sections were rinsed with 0.1 M sodium cacodylate (pH 6.9) buffer until all traces of the yellow osmium carbosilazone (Sigma-Alrich, Cat. M1404) for 14 h at 60 °C in an oven. Sections were analyzed with a Tecnai 20 High Voltage EM (FEI) operating at 200 kV.

Stochastic simulation. A one-dimensional lattice was used to mimic the ER. As schematically represented in Fig. 2g, we divided the lattice in three segments: the front (L₁), the nucleus (L₀) and the back (L₂). The NE was defined as the single voxels between the front (NEf) and the nucleus or the back (NEb) and the nucleus respectively. The time step of the simulation, Δt, was fixed to 1 s, then the dimension of the voxels of the lattice, x, was computed accordingly to the relation: Δt = x²/2D, where D is the diffusion coefficient. We assumed the diffusion coefficient of EMD in the ER to be similar to the one of GFP: 10⁻⁸ m²/s. The number of voxels in the lattice was determined in order to have the lattice length equal to the typical experimental one: 80 μm. We assumed that in total in the ER is arriving 1 EMD molecule per second and that the mean-life of the protein is 50h. We used these values to compute the probability per voxel and per unit of time (Kon) that a molecule is degraded. Molecules in the front and back could freely move, but because EMD interacts with other elements of the NE that stabilized it, we roughly assumed the diffusion coefficient of EMD at the NE neglectable. We started the simulation with a number of EMD molecules at the steady-state and uniformly distributed in the ER, then molecules were allowed to move. At each time step new molecules were generated at both sides of the ER with equal probability Kon and in the ER, as well as at the NE, with probability Kon, molecules were degraded. In Supplementary Movie 2 we show the temporal evolution of the simulation with proportion between L₂ and L₁ similar to the experimental data: L₂ ≈ L₁ ≈ L₀. In Fig. 2g, we show the results of different simulations and we plotted the ratio between the number of EMD molecules at the NE and NEM in function of different values of L₂/(L₂ + L₀) and we compared it with a mathematical model. Our simulations support the idea that an asymmetric distribution of ER in the front versus the back of the cell is sufficient to generate an asymmetric distribution of elements at the NE. A detailed mathematical model is described in Supplementary Text.

Data availability
The authors declare that the data supporting the findings of this study are available in the Supplementary Information files or from the corresponding author upon reasonable request. The source data underlying Figs. 2d, 4b, 4d, 5c and Supplementary Figs. 2a, 2i, 3c, 4c, 4g, 5a, 5b, 6a, 6b, 6c, 7a, 7l are provided as Source Data file.

Code availability
Custom-built ImageJ macros are available from the corresponding author upon request.

Received: 8 July 2019; Accepted: 2 April 2020; Published online: 01 May 2020

References
1. Li, R. & Gundersen, G. G. Beyond polymer polarity: How the cytoskeleton builds a polarized cell. Nat. Rev. Mol. Cell Biol. 9, 860–873 (2008).
12. Parker, K. K. et al. Directional control of lamellipodia extension by Cdc42, MRCK, myosin, and actin flow establishes MOTC polarization in migrating cells. *Cell 121*, 451–463 (2015).

13. Pouthas, F. et al. In migrating cells, the Golgi complex and the position of the centrosome depend on geometrical constraints of the substratum. *J. Cell Sci. 121*, 2406–2414 (2008).

14. Ghosh, S., Stoyanova, V. et al. in Cell Polarity: Biological Role and Basic Mechanisms (ed. Ebnert, K.) 115–146 (Springer International Publishing, 2015).

15. Maiuri, P. et al. The nuclear envelope protein lamin A/C phosphorylation and turnover with feedback to actomyosin. *Curr. Biol. 24*, 1909–1917 (2014).

16. Lattanzi, G. et al. Association of emerin with nuclear and cytoplasmic actin is regulated in differentiating myoblasts. *Biochem. Biophys. Res. Commun. 303*, 764–770 (2003).

17. Holaska, J. M., Kowalski, A. K. & Wilson, K. L. Emerin caps the pointed end of actin filament: evidence for an actin cortical network at the nuclear inner membrane. *PLoS Biol. 2*, e231 (2004).

18. de Lanerolle, P. Nuclear actin and myosins at a glance. *J. Cell Sci. 125*, 1945–1949 (2012).

19. Cremers, T. & Cremers, C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat. Rev. Genet. 2*, 292–301 (2001).

20. Kind, J. et al. Single-cell dynamics of genome-nuclear lamina interactions. *Cell 153*, 178–192 (2013).

21. van Steensel, B. & Belmont, A. S. Lamina-associated domains: links with chromosome architecture, heterochromatin, and gene repression. *Cell 169*, 780–791 (2017).

22. Berget, M. et al. Confoal reference free traction force microscopy. *Nat. Commun. 7*, 12814 (2016).

23. DiMilla, P. A. et al. Maximal migration of human smooth muscle cells on fibronectin and type IV collagen occurs at an intermediate attachment strength. *J. Cell Biol. 122*, 729–737 (1993).

24. Callan-Jones, A. et al. Confinement and low adhesion induce fast amoeboid migration of slow mesenchymal cells. *Cell 160*, 659–672 (2015).

25. Bonne, G. et al. Lamin and nespin-1 mediate inside-out mechanical coupling in muscle cell precursors through FHO1. *Sci. Rep. 7*, 1–14 (2017).

26. Pfaf, J. et al. Emery-Dreifuss muscular dystrophy mutations impair TRC40-mediated targeting of emerin to the inner nuclear membrane. *J. Cell Sci. 129*, 502–516 (2016).

27. Cooper, J. A. Effects of cytolalsin and phallolidin on actin. *J. Cell Biol. 105*, 1473–1478 (1987).

28. Straight, A. F. et al. Dissecting temporal and spatial control of cytokinesis with a myosin II inhibitor. *Science 299*, 1743–1747 (2003).

29. Fabian, L., Trosic, J., J. & Forer, A., Calyculin A, an enhancer of myosin, speeds up anaphase chromosome movement. *Cell Chromosom 6*, 42–45 (2007).

30. Vasquez, R. J., Howell, B., Yvon, A. M. C., Wadsworth, P. & Cassimeris, L. Nanomolar concentrations of nucodazole alter microtubule dynamic instability in vivo and in vitro. *Mol. Biol. Cell 8*, 973–985 (1997).

31. Biene, S., Maestrelli, E. & Rivella, S. Dreius muscular dystrophy. *Nat. Genet. 8*, 323–327 (1994).

32. Janin, A., Bauer, D., Ratti, F., Millat, G. & Mest, A. Nuclear envelopes: a complex LINC between nuclear envelope and pathology. *Orphanet J. Rare Dis. 12*, 1–16 (2017).

33. Chang, W., Worman, H. J. & Gundersen, G. G. Emerin associates actin flow for nuclear movement and centrosome orientation in migrating fibroblasts. *Mol. Biol. Cell 24*, 3869–3880 (2013).

34. Perovanic, J. et al. Laminopathies disrupt epigenomic developmental programs and cause complex mosaicism for multifunctionality. *J. Cell Biol. 208*, 11–22 (2015).

35. Lammert, J. et al. Abnormal nuclear shape and impaired mechanotransduction in emerin-deficient cells. *J. Cell Biol. 170*, 781–791 (2005).

36. Duong, N. T. et al. Nesprins: tissue-specific expression of epsilon and other short isoforms. *PLoS ONE 9*, e94380 (2014).

37. Hebling-Leclerc, A., Bonne, G. & Schwartz, K. Emery-Dreifuss muscular dystrophy. *Eur. J. Hum. Genet. 10*, 157–161 (2002).

38. Solovei, I., Cavallo, A. & Schermelleh, L. Spatial preservation of nuclear chromatin architecture during three-dimensional fluorescence in situ hybridization (3D-FISH). *Exp. Cell Res. 23*, 10–23 (2002).

39. Zuleger, N. et al. System analysis shows distinct mechanisms and common principles of nuclear envelope protein dynamics. *J. Cell Biol. 193*, 109–123 (2011).

40. Lombardi, M. L. et al. The interaction between nesprins and sun proteins at the nuclear envelope is critical for force transmission between the nucleus and cytoskeleton. *J. Cell Biol. 286*, 26743–26753 (2011).

41. Shao, S., Irianto, J. & Discher, D. E. Mechanosensing by the nucleus: From pathways to scaling relationships. *J. Cell Biol. 216*, 303–315 (2017).

42. Kirby, T. J. & Lammert, J. Emerging views of the nucleus as a cellular mechanosensor. *Nat. Cell Biol. 20*, 373–381 (2018).

43. Zhu, R., Antoku, S. & Gundersen, G. G. Centriolar displacement of nucleus reveals multiple LINC complex mechanisms for homeostatic nuclear positioning. *Curr. Biol. 27*, 3097–3110.e3 (2017).

44. Davidson, P. M. et al. Actin accumulates Nesprin-2 at the front of the nucleus during confined cell migration. *SSRN Electron. J.* [https://doi.org/10.2139/ssrn.3426657] (2019).

45. Mislow, J. M. K. et al. Nesprin-1a self-associates and binds directly to emerin and lamin A in vitro. *FEBS Lett. 525*, 135–140 (2002).

46. Ho, C. Y. & Lammert, J. Lamins at a glance. *J. Cell Sci. 125*, 2087–2093 (2012).
Acknowledgements
This work was supported by Italian Association for Cancer Research (AIRC) fellowships for Paulina Nastał (Project code: 19416) and Gururaj Rao Kidiyoor (Project code: 19464). This project was funded by Fondazione Umberto Veronesi Post-doctoral fellowships for Paulina Nastał. The authors would like to thank IFOM Imaging Facility and IFOM Cell Biology Unit for help with performing experiments. We would like to acknowledge all the participants to the Milano 4D-Nucleus meetings, Giorgio Scita, Nils Gauthier, Matthieu Piel, and Kristina Havas Cavalletti for helpful comments and discussions. Martina Galli and Ylli Doksani for generation of pLEO-EMD-GFP plasmid. Bas van Stensel, Marloes van der Zwalm, and Daniel Peric Hupkes for sharing with us the DamID plasmids. We would like to acknowledge Shivashankar GV for DN-KASH-EGFP plasmid. We thank Dr. Marina Mora from the “C.Besta” IRCCS Neurological Institute and Telethon Biobank for the primary myoblasts samples.

Author contributions
P.M. designed experiments and supervised the study. P.N. designed, carried out and interpreted experiments. D.F.P. and A.P. performed biochemical experiments. G.R.K. performed live cell imaging with EMD-EGFP cells. S.M. performed chemotaxis and myoblast infection experiment. T.L., D.P., and A.F. supported this project with TFM and analysis. G.V.B. and A.M. performed TEM. O.M.R. and M.C.L. developed the mathematical model. S.I. helped with generation of stable cell line P.M. and P.N. carried out data and statistical analysis. P.M. and P.N. wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-15910-9.

Correspondence and requests for materials should be addressed to P.M.

Peer review information Nature Communications thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020