Morphological polarization involving changes in cell shape and redistribution of cellular signaling machinery, initiate the migration of mammalian cells. Golgi complex typically localizes in front of the nucleus, and this frontwards polarization has been proposed to be involved in directional migration. However, the sequence of events remains unresolved. Does Golgi polarization precede directional migration or vice-versa? We address this question by constraining cells to specific areas and shapes then tracking their motile behavior and the spatio-temporal distribution of Golgi apparatus upon release. Results show that while the position of the Golgi complex depends on the cell geometry, the subcellular localization of the Golgi complex does not define the cell's leading edge. Cells constrained within elongated geometries exhibit polarized extension of lamellipodia and upon release, migrate preferentially along the long axis of the cell. Minimally constrained cells released from larger areas however, exhibit retarded migration regardless of lamellipodia protrusion activity.

Cell motility is critical in tissue formation, wound healing and the engineering functional tissues in vitro. The movement of individual cells is initiated by morphological polarization, manifested by morphological changes and redistribution of intracellular components. In migrating cells, calcium micro domains1,2, pH domains3,4, cytoskeletal components5,6, centrosomes, lamellipodium, microtubule organization center (MTOC), and Golgi apparatus typically distribute toward the direction of migration7,8. Directional protrusions, driven by actin polymerization at the leading edge, form lamellipodia that attach to the extracellular matrix (ECM) on the substrate9. Cells attach to the ECM more tightly at the leading edge than the rear of the cell, and this gradient in adhesive force generates traction for movement10,11.

Cell-ECM adhesion strength has been shown to influence the speed of cell movement12–14. Systematic variations in local cell-ECM adhesion strength have been achieved by varying integrin expression levels15, integrin clustering16, integrin-ECM binding affinities17, and surface ECM concentrations18,19. In all of these studies, cells are observed to be most motile at intermediate local cell-ECM adhesive strengths. These studies clearly demonstrate the role of cell-ECM adhesion strength on cell migration. However, alterations in cell-ECM adhesion strength in these studies, simultaneously changes cell spreading area, which alters the cells’ mechanical force balance20,21. It is unknown whether the migration dependence on cell ECM adhesions observed is caused by alterations in cell spreading, integrin binding, or both.

A population of a single cell type invariably exhibits a broad distribution of spreading areas. For example, a population of keratocytes of the same origin on the same substrate exhibit spreading areas ranging from 100–1000 μm². Likewise, NIH 3T3 fibroblasts adopt spreading areas ranging from 900–2500 μm² on tissue culture dishes. Cell spreading also changes for individual cells as they migrate, with membrane extensions at the leading edge and retraction of the rear cyclically increasing and decreasing the spreading area. Studies with micropatterned cells have shown that cell spreading influences cell proliferation21,22, focal adhesion assembly23, gene expression and protein synthesis24. However, the effects of cell spreading area, independent of cell morphology and local cell-ECM interactions, on individual cell motility in populations exhibiting broad distributions of spreading areas remains unknown.

It has been proposed that subcellular localization of the Golgi apparatus and MTOC relative to the cell nucleus represent the polarity of cells, with the Golgi and MTOC typically oriented toward the leading edge25. This is supported by consistent observations of Golgi apparatus and MTOC toward the leading edge in migrating fibroblasts26,27, endothelial cells28, and neurons29. The front position of the Golgi apparatus and MTOC has been shown to play a crucial role in directed migration30 providing membranes and associated proteins to facilitate forward protrusions31,32. Inhibition of the frontal location of MTOC or Golgi interferes with directed cell migration toward the wound in scratch wound assays33–35. Cdc42 has also been shown to be the key determinant in establishing the front position of the Golgi and/or MTOC36,37. Although these observations show that Golgi polarization accompanies directional migration, the sequence of events has not been addressed. Does directional
migration arise from the polarization of Golgi relative to the nucleus or the up-regulation of lamellipodia extension at the leading edge?

Here we address these fundamental questions using micropatterned polyelectrolyte self-assembly\textsuperscript{38,39} to first regulate the size and shape of individual cells then non-invasively releasing them from confinement to observe their subsequent migratory behavior.

**Results**

**Cell confined within smaller areas have greater migration tendency upon release.** To examine the effects of cell spreading area on motility, we first confine NIH 3T3 fibroblasts onto cell adhesive islands on tissue culture dishes defined by microcontact printing surrounding regions with cell-resistant poly(OEGMA/
MA). Within 12 hours, 3T3 fibroblasts spread to assume the size and shape of the 900, 1600, and 2500 µm² rectangular islands, designed to mimic the elongated spindle-shaped morphology of fibroblasts observed in vivo.26 After defining the size and shape of the cells, we released them from confinement by adding media-soluble chitosan39 which gets adsorbs onto the cell-resistant poly(OEGMA/MA) rendering these surrounding regions cell-adhesive. Cells confined in 900 µm² or 1600 µm² rectangles spread to fully occupy the available area, whereas cells confined within 2500 µm² rectangles spread to areas typically 10% smaller than the constraining rectangle. The relationship between cell migration tendency and initial spreading area is shown in Figure 1 for NIH 3T3 cells confined to similar rectangles of varying area. Irrespective of their confined area, cells all exhibit active lamellipodia extension at the corners of the rectangles. However, only cells from 900 µm² adhesive islands move out of confinement within 12 hours after release. We quantified the effect of initial cell area on cell movement upon release by tracking cell areas within and outside the initially confining rectangles over time. As Figure 1b shows, cells with initial area of 900 µm² retain almost no portion of their body within the initial region of confinement after 12 hours, indicating migration out of the confinement. In contrast, cells with initial confinement areas of 1600 and 2500 µm² retain full coverage of their initial region of confinement. The increased cell area outside the pattern is due to overall cell spreading as the increased in total area shows. NIH 3T3 fibroblasts initially confined within 900 µm² islands of other geometries also exhibit greater migration tendencies when released compared to cells released from 2500 µm² islands of the same geometry (Supplementary Figure S1).

These results show that spreading area alone can influence motility with smaller spreading areas correlated with higher cell motility. In contrast to earlier reports, the cell shape and areas are modulated here without altering the local interaction between the cell and substrate prior to release. Earlier conclusions of reduced average motility when the majority of Golgi orient toward the long edge of the rectangle (Figure 3c). This preference increases with increasing cell spreading area with 82% of 2500 µm² cells orienting their Golgi apparatus toward the long edge of the cell (Figure 3b). The splitting of Golgi apparatus to both short edges occurred on rare occasions (observed for 2 of 93 and 3 of 70 cells for 900 µm² and 2500 µm² rectangles, respectively) and these were not included in the analysis.

To examine the localization of Golgi apparatus, the angle over which Golgi is distributed and quantified for all cells with either short edge (S) or long edge (L) orientation. As Figure 3d shows, the Golgi apparatus cluster over more compact spread angles for cells with smaller spreading areas. These results show that cell size and shape influence the distribution of Golgi. Cell elongation polarizes the Golgi apparatus toward the long edge while cell area impacts the localization of Golgi apparatus. Since the Golgi apparatus is generally located in front of the nucleus towards the leading edge of migratory cells,45 the initial direction of cell movement upon release may be expected to be directed horizontally outwards from the long edges.

**Geometric confinement influences Golgi apparatus polarization and orientation.** The position of the Golgi apparatus relative to the nuclei has been proposed to indicate cell polarity with the Golgi apparatus oriented towards the leading edge25. Cell shape alone has been shown to influence the polarization of mammalian cells on teardrop-shaped adhesive islands, with polarization towards the blunt end.6,45,46 However, the independent role of cell spreading area and cell morphology on polarization of Golgi apparatus remains unanswered.

To quantify the effects of cell spreading areas and geometry on Golgi orientation, we analyzed the distribution of Golgi in fibroblast cells confined within 900, 1600 and 2500 µm² rectangular islands (Figure 3a, b). Cells within the rectangular islands adopt the elongated confining geometry with an average aspect ratio 2.5 ± 0.3. We divided the periphery of the nucleus by two intersecting perpendicular diagonal lines centered at the centroid of the nucleus and classified the location of Golgi apparatus as S when the majority of Golgi orient toward the short edge, L when the majority of Golgi orient toward on the long edge, O when the majority of the Golgi overlaps with the nucleus. Cells with different spreading areas preferentially orient their Golgi apparatus toward the long edge of the rectangle (Figure 3c). This preference increases with increasing cell spreading area with 82% of 2500 µm² cells orienting their Golgi toward the long edge. Cells with the Golgi apparatus oriented toward the short edge exhibit atypical nuclear alignment with the long axis of the nucleus orienting more perpendicularly to the long axis of the cell (Figure 3b). The splitting of Golgi apparatus to both short edges occurred on rare occasions (observed for 2 of 93 and 3 of 70 cells for 900 µm² and 2500 µm² rectangles, respectively) and these were not included in the analysis.

**Directional membrane protrusions mediate initial movement.** To investigate whether the increased bias of Golgi towards the long edge versus the short edge lead to preferential migration from the long edge, cells were released by rendering the background region cell adhesive through adsorption of chitosan added to the media39. Before release, cells confined within elongated patterns align their actin cytoskeleton diagonally or along the long axis of the cell body (Figure 4a). Although the Golgi of the confined cells are predominantly located close to one of the long edges of 900 µm² rectangles (48%, Figure 3c) compared to the short edge (32%, Figure 3c), we observed that cells extend lamellipodium predominantly from...
the corners 4 hours after release (Figure 4a). Tracking the centroid of individual fibroblasts 4, 8, and 12 hours after release (Figure 4b) reveals that this biased extension of lamellipodia leads to predominantly vertical translocation wherein cells move out principally from one of the short edges of the rectangles. These findings show that the initial elongated cell shape influences the location of the initial membrane protrusions and biases the direction of initial cell movement along the long axis.
The Golgi apparatus reorients upon release from elongated confinement. Cells released from rectangular confinement typically migrate along their elongated axis with Golgi on one side of the nucleus instead of in front towards the leading edge. When released from smaller areas (900 µm² and 1600 µm²), Golgi apparatus reorient to the front of the nuclei towards the short edge where active membrane protrusions are leading the cell forwards (Figure 5a, b). However for cells released from larger rectangular islands (2500 µm²), which exhibit negligible movement out of the pattern, 20 of 27 cells analyzed repositioned their Golgi around the nuclei without establishing a defined polarization (Figure 5c) while the remainder exhibited no repositioning of Golgi (Figure 5d). Integration of the corresponding beta distribution yields an unbiased probability (defined here to be one where cells released from the 2500 µm² islands reposition their Golgi without a defined polarization between 45% and 55% of the time) of 2.3%. These observations show that the Golgi polarity observed in cells does not necessarily determine the directional cell movement, but rather is the result of active cell movement.

Discussion

Cell shape and spreading have been known to influence the mechanical force balance within mammalian cells²⁰,²¹. Here, we released cells of defined size and shape and demonstrated that cell spreading area alone influences significantly the rate of cell movement upon release. There is a difference between the substrate that the cells are initially confined, i.e., cell culture dish, and the substrate that cells subsequently migrate onto, chitosan, which tempers this finding. The commonly used wound healing assay to measure cell migration rate is a useful tool for studying collective cell movements wherein the average spreading area of cells can also be controlled via the seeding density. However, cell-cell contacts influence the polarity of individual cells⁵⁰ and the method is unsuitable for investigating the polarity and movement of individual cells. The micropatterned polyelectrolyte self-assembly method used here to temporally and spatially control cell dimensions provides a tool for understanding the coordinated regulation of cell geometry and movement of individual cells.
Here, confined cells were released from defined starting geometries to visualize the time-dependent Golgi reorganization and directional protrusions during confinement and after release. Our results show that cells with limited spreading area polarize and migrate out of confinement more rapidly than cells with larger initial spreading area. Fibroblasts released from elongated confinement, which mimic their morphology in vivo8,9, show that the position of Golgi is dependent on spreading area and morphology of the cell. However, the position of Golgi relative to the nucleus prior to the release does not correspond to the direction of movement upon release. Instead, lamellipodia, which extend more actively from the short edges, consistently define the majority of cell movements along the long axis of the cells.

Only after cells migrate out from confinement does the Golgi apparatus reorient toward the direction of cell movement. This is consistent with previous findings that Golgi apparatus locate toward the leading edge of migrating fibroblasts and neurons20,29,30. The final front position of the Golgi apparatus provides membranes and proteins from Golgi-derived vesicles necessary to sustain forward movement23,36,37.

Earlier experiments with cells confined and released from asymmetric teardrop shapes have suggested that asymmetric geometries can lead to cell polarization as indicated by the localization of the Golgi in front of the blunt edge of the teardrops from where migration initiates48. With the current rectangular-shaped patterns, we observed reorientation of Golgi towards the direction of migration. These data suggest that actin organization resulting from the teardrop morphology, rather than Golgi polarization, can lead to the observed asymmetric lamellipodia extension and directional migration.

The results of these controlled experiments on Golgi reorientation dynamics following initial migration imply that Golgi polarization in front of the nucleus is not a prerequisite for directional migration. This idea is supported by a few studies on free migrating cells10, and cells migrating along line patterns42 that exhibit uncorrelated localization of Golgi and/or MTOC with the migration direction. Cell geometry sets the actin filament organization and membrane protrusions that determine the direction of initial cell movement independent of the position of Golgi. The initial Golgi position does not define the leading edge. Instead, reorientation follows directional movement and the final frontal position suggests that it may be important for maintaining but not initiating directional migration. The time dependent polarization of Golgi apparatus highlights the complex coordination of intracellular structures in response to changes in their extracellular environments. These results motivate more detailed investigation of the spatial and temporal interactions of the intracellular structures and signals in mediating polarity, a process important during morphogenesis and wound healing.

Methods

Materials. Tissue culture dishes were purchased from Fisher Scientific (catalog no. 430166) and used as received. Polydimethylsiloxane (PDMS; Sylgard 184) was obtained from Dow Corning (Midland, MI) and used at 10 : 1 (w : w) base: curing agent. Media-soluble chitosan (93 kDa, 74% deacetylation) was a gift from Tri Corporation (Alpharetta, GA). Alexa 488 or 594-phalloidin, 4′,6-diamidino-2-phenylindole (DAPI), golgin-97 (human), mouse IgG1, monoclonal CDF4 (anti-Golgi), CellLight Golgi-RFP *BacMam 2.0, and Alexa Fluor® 488 goat anti-mouse IgG1 (γ1) (secondary antibody) were purchased from Molecular Probes (Eugene, OR). Phosphate buffered saline (PBS), IScove’s modified Dulbecco’s medium (IMDM), and Fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). Platelet- Derived Growth Factor-BB human (PDGF) was obtained from Sigma-Aldrich (St. Louis, MO).

Preparation of poly(OEGMA-co-MA). Random copolymers of oligo(ethylene glycol) methacrylate (OEGMA) and methacrylic acid (MA) (Scientific Polymer Products, NY) were prepared by free radical polymerizations of 10 wt% methanolic solutions of the two monomers (80 : 20 OEGMA to MA mass ratio) at 60 °C over 24 hrs, initiated with 1 wt% (with respect to monomer) 2′,2′-azobis(2- amidinopropane) dihydrochloride (Wako, VA).

Cell confinement and release. Micropatterns of varying size (900, 1600, and 2500 μm²) and shapes (square, triangle, pentagon, hexagon, and trapezoid) were fabricated on silicon wafers using standard photolithographic techniques. From this silicon master, complementary PDMS replicas were prepared by soft lithography33 and used as stamps in subsequent microcontact printing steps to form patterns of poly(OEGMA-co-MA) copolymer directly on cell culture dishes. Patterned dishes were sterilized under UV for one hour prior to plating of cells. Masking of poly(OEGMA-co-MA) printed regions by adsorption of chitosan to render these regions cell-adhesive was accomplished by immersing the culture dish with media containing 0.2% water-soluble chitosan for 20 minutes35. All experiments were designed only to change the geometric confinement of the cells. All other conditions including the nutrient or chitosan properties were maintained constant.

Cell culture. NIH 3T3 fibroblasts were purchased from ATCC and cultured in IScove’s modified Dulbecco’s medium (IMDM) supplemented with 10% FBS. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO2. Sub-confluent mononuclears were dissociated with 0.25% trypsin-EDTA solution, re-suspended in IMDM with 10% FBS, and then plated on micropatterned culture dishes.

Immunofluorescence staining. Cells were fixed with 3.7% paraformaldehyde for 15 minutes, washed in phosphate buffered saline, permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 3% BSA (bovine serum albumin) for 30 minutes. Samples were subsequently rinsed with PBS then incubated with Alexa 594-phalloidin, Alexa 488 conjugated anti-Golgin-97 (human), and DAPI to stain for F-actin, Golgi apparatus, and nuclei respectively. Fixed cells were imaged using Zeiss LSM 710 NLO confocal microscope, 63× plan apochromatic water objective lens and digitally acquired using Zeiss Meta software version 3.5.

Cell migration and statistical analysis. Cells were plated at a density of ~10,000 cells/cm² and allowed to spread within the cell-adhesive patterns of different sizes simultaneously for 12 hours. The cells were then released from confinement by incubating with complete media containing 0.2% chitosan for 20 minutes, after which the cells were restored in complete media containing no chitosan and with 5 ng/ml PDGF. Migration following release was tracked with Nikon TE-2000 inverted microscope equipped with CCD camera (SPOT CAM, Diagnostic Instruments Inc.), 20×, NA 0.25 A-plan objective lens. Time-lapse phase contrast images were processed with Metamorph software (Ver 6.0r4, Universal Imaging, Westchester, PA). Only individual cells confined within each individual island were considered for analysis. The cell migration out of the pattern was tracked for 12 hours beyond which cells divisions were observed. Quantification of cell shape and determination of the location of the Golgi were performed using NIH Image and Metamorph software. Statistical analysis on the localization of Golgi toward either the short or long edges were performed assuming a binominal distribution. Significance analysis of angular distributions of Golgi apparatus for fibroblast cells on rectangular patterns with different areas were analyzed taking into account differences in sample variances, i.e., heteroscedastic t-test.

1. Brundage, R. A., Fogarty, K. E., Tuft, R. A. & Fay, F. S. Calcium gradients underlying polarization and chemotaxis of eosinophils. Science 254, 703–706 (1991).
2. Wei, C. et al. Calcium flickers steer cell migration. Nature 457, 901–905 (2009).
3. Servant, G. et al. Polarization of chemoattractant receptor signaling during neutrophil chemotaxis. Science 287, 1037–1040 (2000).
4. Jin, T., Zhang, N., Long, Y., Parent, C. A. & Devreotes, P. N. Localization of the G protein beta gamma complex in living cells during chemotaxis. Science 287, 1034–1036 (2000).
5. Schmidt, C. E., Horwitz, A. F., Lauffenburger, D. A. & Sheetz, M. P. Integrin-cytoskeletal interactions in migrating fibroblasts are dynamic, asymmetric, and regulated. J. Cell Biol. 123, 977–991 (1993).
6. Lawson, M. A. & Maxfield, F. R. Ca²⁺ and calcinurin-dependent recycling of an integrin to the front of migrating neutrophils. Nature 377, 75–79 (1995).
7. Nabi, I. R. The polarization of the motile cell. J. Cell Sci. 112, 1803–1811 (1999).
8. Lauffenburger, D. A. & Horwitz, A. F. Cell migration: A physically integrated molecular process. Cell 84, 359–369 (1996).
9. Theriot, J. A. & Mitchison, T. J. Actin microfilament dynamics in locomoting cells. Nature 352, 126–131 (1991).
10. Stossel, T. P. On the crawling of animal cells. Science 260, 1086–1094 (1993).
11. Laxer, D. A., Sandberg, R. R. & Horwitz, A. F. Cell adhesion in cell-migration. Curr. Opin. Struct. Biol. 7, 697–706 (1995).
12. Dimilla, P. A., Barbee, K. & Lauffenburger, D. A. Mathematical model for the effects of adhesion and mechanics on cell migration speed. Biophys. J. 60, 15–37 (1991).
13. Mandeville, J. T. H., Lawson, M. A. & Maxfield, F. R. Dynamic imaging of neutrophil migration in three dimensions: Mechanical interactions between cells and matrix. J. Leukocyte Biol. 61, 188–200 (1997).
14. Palecek, S. P., Lofus, J. C., Ginsberg, M. H., Lauffenburger, D. A. & Horwitz, A. F. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature 385, 537–540 (1997).
15. Giancotti, F. G. & Ruoslahti, E. Elevated levels of the alpha 5 beta 1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. *Cell* 60, 849–859 (1990).

16. Maheshwari, G., Brown, G., Lauffenburger, D. A., Wells, A. & Griffith, L. G. Cell adhesion and motility depend on nanoscale RGD clustering. *J. Cell Sci.* 113, 1677–1686 (2000).

17. Kuipers, T. W. et al. Freezing adhesion molecules in a state of high-avidity binding blocks eosinophil migration. *J. Exp. Med.* 178, 279–284 (1993).

18. Zimina, M., Greaves, T. & Gabriele, S. Spatial coordination between cell and nuclear shape within micropatterned endothelial cells. *Nat. Commun.* 3, 671 (2012).

19. Chen, C. S., Mrksich, M., Huang, S., Whitesides, G. M. & Ingber, D. E. Geometric activation, detected using a novel assay, mediates microtubule organizing center assembly in cultured fibroblasts at the edge of an experimental wound. *Proc. Natl. Acad. Sci. USA* 79, 2603–2607 (1982).

20. Schaar, B. T. & McConnell, S. K. Cytoskeletal coordination during neuronal migration. *Proc. Natl. Acad. Sci. USA* 102, 13652–13657 (2005).

21. Itoh, R. et al. Activation of Rac and Cdc42 video imaged by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. *Cell. Mol. Biol. Cell* 22, 6582–6591 (2002).

22. Bergmann, J. E., Kupfer, A. & Singer, S. J. Membrane insertion at the leading edge of motile fibroblasts. *Proc. Natl. Acad. Sci. USA* 80, 1367–1371 (1983).

23. Ridley, A. J. et al. Cell migration: Integrating signals from front to back. *Science* 302, 1704–1709 (2003).

24. Euteneuer, U. & Schlwa, M. Mechanism of centrosome positioning during the wound response in BSC-1 cells. *J. Cell Biol.* 116, 1157–1166 (1992).

25. Gomes, E. R., Jani, S. & Gundersen, G. G. Nuclear movement regulated by Cdc42, MBCK, myosin, and actin flow establishes MTOC polarization in migrating cells. *Cell* 121, 451–463 (2005).

26. Gotlieb, A. I., Subrahmanyan, L. & Kalnins, V. I. Microtubule-organizing centers and cell migration: effect of inhibition of microtubule and microtubule disruption in endothelial cells. *J. Cell Biol.* 96, 1266–1272 (1983).

27. Etienne-Manneville, S. & Hall, A. Rho GTPases in cell biology. *Nature* 420, 629–635 (2002).

28. Co, C. C., Wang, Y. C. & Ho, C. C. Biocompatible micropatterning of two different cell types. *J. Am. Chem. Soc.* 127, 1598–1599 (2005).

29. Kumar, G., Meng, J. J., Ip, W., Co, C. C. & Ho, C. C. Cell motility assays on tissue culture dishes via non-invasive confinement and release of cells. *Langmuir* 21, 9267–9273 (2005).

30. Li, F., Li, B., Wang, Q.-M. & Wang, J. H. C. Cell shape regulates collagen type I expression in human tendon fibroblasts. *Cell Motil. Cytoskel.* 65, 332–341 (2008).

31. Pelham, R. J. & Wang, T. L. C. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. USA* 94, 13661–13665 (1997).

32. Parker, K. et al. Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *FASEB J.* 16, 1195–1204 (2002).

33. Naumann, P., Lappalainen, P. & Hotulainen, P. Mechanisms of actin stress fibre assembly. *J. Microsc.-Oxford* 231, 446–454 (2008).

34. Bhadriraju, K. & Hansen, L. K. Extracellular matrix-and cytoskeleton-dependent changes in cell shape and stiffness. *Exp. Cell Res.* 278, 92–100 (2002).

35. Doornaert, B. et al. Time course of actin cytoskeleton stiffness and matrix adhesion molecules in human bronchial epithelial cell cultures. *Exp. Cell Res.* 287, 199–208 (2003).

36. Chrzanoswka-Wodnicka, M. & Burridge, K. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J. Cell Biol.* 133, 1403–1415 (1996).

37. Jiang, Y. J., Bruzewicz, D. A., Wong, A. P., Piel, M. & Whitesides, G. M. Directing cell migration with asymmetric micropatterns. *Proc. Natl. Acad. Sci. USA* 102, 975–978 (2005).

38. Kumar, G., Co, C. C. & Ho, C. C. Steering cell migration using microarray amplification of natural directional persistence. *Langmuir* 27, 3803–3807 (2011).

39. Desai, R. A., Gao, L., Raghavan, S., Liu, W. F. & Chen, C. S. Cell polarity triggered by cell-cell adhesion via E-cadherin. *J. Cell Sci.* 122, 903–911 (2009).

40. Danowski, B. A., Khodjakov, A. & Wadsworth, P. Centrosome behavior in motile HGF-treated PtK2 cells expressing GFP-gamma tubulin. *Cell Motil Cytoskel.* 50, 59–68 (2001).

41. 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).

42. Qin, D., Xia, Y. & Whitesides, G. M. Soft lithography for micro- and nanoscale patterning. *Nat. Protoc.* 5, 491–502 (2010).

43. Kumar, G., Meng, J. J., Ip, W., Co, C. C. & Ho, C. C. Cell motility assays on tissue culture dishes via non-invasive confinement and release of cells. *Langmuir* 21, 9267–9273 (2005).

44. Li, F., Li, B., Wang, Q.-M. & Wang, J. H. C. Cell shape regulates collagen type I expression in human tendon fibroblasts. *Cell Motil. Cytoskel.* 65, 332–341 (2008).

45. Pelham, R. J. & Wang, T. L. C. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. USA* 94, 13661–13665 (1997).

46. Parker, K. K. et al. Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *FASEB J.* 16, 1195–1204 (2002).

47. Naumann, P., Lappalainen, P. & Hotulainen, P. Mechanisms of actin stress fibre assembly. *J. Microsc.-Oxford* 231, 446–454 (2008).

48. Bhadriraju, K. & Hansen, L. K. Extracellular matrix-and cytoskeleton-dependent changes in cell shape and stiffness. *Exp. Cell Res.* 278, 92–100 (2002).

49. Doornaert, B. et al. Time course of actin cytoskeleton stiffness and matrix adhesion molecules in human bronchial epithelial cell cultures. *Exp. Cell Res.* 287, 199–208 (2003).

50. Chrzanoswka-Wodnicka, M. & Burridge, K. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J. Cell Biol.* 133, 1403–1415 (1996).

51. Jiang, Y. J., Bruzewicz, D. A., Wong, A. P., Piel, M. & Whitesides, G. M. Directing cell migration with asymmetric micropatterns. *Proc. Natl. Acad. Sci. USA* 102, 975–978 (2005).

52. Kumar, G., Co, C. C. & Ho, C. C. Steering cell migration using microarray amplification of natural directional persistence. *Langmuir* 27, 3803–3807 (2011).

53. Desai, R. A., Gao, L., Raghavan, S., Liu, W. F. & Chen, C. S. Cell polarity triggered by cell-cell adhesion via E-cadherin. *J. Cell Sci.* 122, 903–911 (2009).

54. Danowski, B. A., Khodjakov, A. & Wadsworth, P. Centrosome behavior in motile HGF-treated PtK2 cells expressing GFP-gamma tubulin. *Cell Motil Cytoskel.* 50, 59–68 (2001).

55. 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).

56. Qin, D., Xia, Y. & Whitesides, G. M. Soft lithography for micro- and nanoscale patterning. *Nat. Protoc.* 5, 491–502 (2010).
Author Correction: Geometric Control of Cell Migration

Bo Chen¹, Girish Kumar¹,², Carlos C. Co¹ & Chia-Chi Ho¹

Correction to: Scientific Reports https://doi.org/10.1038/srep02827, published online 3 October 2013

The following information was omitted from the Methods section.

The images in Figures 2, 3B, and 4A were captured through a coverslip. To achieve this, we cut the boundary of tissue culture dishes and mounted with fluoromount-G. The contrast/brightness of the Golgi stain in Figure 5 was adjusted using Image J software which can alter certain details of the Golgi structure. However, the key point was to show the brief position of the Golgi apparatus during the release period. They are not relative to the certain details of the Golgi structure.

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) 2018

¹Chemical & Materials Engineering Program, University of Cincinnati, Cincinnati, OH, 45221-0012, USA. ²Division of Biology, Center for Devices and Radiological Health, US Food & Drug Administration, Silver Spring, MD, 20993, USA. Correspondence and requests for materials should be addressed to C.-C.H. (email: hocc@ucmail.uc.edu)