Magnetic nanoparticle–mediated massively parallel mechanical modulation of single-cell behavior

Peter Tseng1,2, Jack W Judy1–3 & Dino Di Carlo1,3,4

We report a technique for generating controllable, time-varying and localizable forces on arrays of cells in a massively parallel fashion. To achieve this, we grow magnetic nanoparticle–dosed cells in defined patterns on micromagnetic substrates. By manipulating and coalescing nanoparticles within cells, we apply localized nanoparticle-mediated forces approaching cellular yield tensions on the cortex of HeLa cells. We observed highly coordinated responses in cellular behavior, including the p21-activated kinase–dependent generation of active, leading edge–type filopodia and biasing of the metaphase plate during mitosis. The large sample size and rapid sample generation inherent to this approach allow the analysis of cells at an unprecedented rate: in a single experiment, potentially tens of thousands of cells can be stimulated for high statistical accuracy in measurements. This technique shows promise as a tool for both cell analysis and control.

Mechanical force has a critical role in many cellular processes, including cell division, contractility, differentiation and motility. The study of how cells respond to, transmit and convert these mechanical signals into chemical signals (mechanotransduction) is a burgeoning field of science1–3; understanding the recurring mechanisms in cellular response to force not only enlightens us about single-cell biology, but it could also provide a tangible method by which to influence cellular function. A fundamental need in the study of cellular mechanics is the on-demand local application of controlled forces over a large population of cells to obtain statistically relevant measurements of noisy biological responses.

Current approaches in which a probing instrument (such as an atomic force microscope tip4–6, optical and magnetic tweezers7–10 or a micropipette11–13) is brought in registration with a single cell are intrinsically limited by the serial nature of single-cell manipulation and the inability to maintain spatially resolved, well-controlled stimuli for prolonged periods of time. Remote approaches, including magnetic twisting cytometry and optical tweezers, typically generate up to 300 pN of force, which is below the 100-nN maximum force that cells can generate. Although bulk approaches including cell stretching14,15, micropost manipulation16 and use of silicon microchips17 can yield larger forces, they are limited by a lack of spatial resolution in mechanical stimulation and the ability to resolve a localized stimulus at the single-cell level. In this work, we demonstrate a hybrid approach in which many individually patterned magnetic nanoparticle–dosed cells are brought into uniform alignment with arrays of magnetizable ferromagnetic elements18. Coalescing of internalized nanoparticles proximal to micromagnetic elements through the application of an external magnetic field allows the generation of highly localized, repeatable mechanical stimuli (in excess of 100 nN and 5 nN µm−1) on the cellular cortex, resolving many of the limitations in throughput, scalability and resolution in existing approaches. This capability comes at the cost of greater system complexity: substrates and magnetic nanoparticles must be specifically designed to achieve an optimal effect. However, designing cellular and micromagnetic patterns gives scientists additional control over the localization and distribution of mechanical stimuli.

We found that mechanical tension mediated by localized nanoparticles in HeLa cells generates a coordinated cellular response in both local biochemistry and higher-order biological processes. Applied stimuli generated substantial asymmetry in filopodia at tensions above 1 nN µm−1 dependent on the activation of the mechanotransductive protein p21-activated kinase (PAK). Finally, we found that asymmetric nanoparticle-mediated forces, applied throughout mitosis, can strongly bias the mitotic spindle axis in a manner that competes with extracellular adhesive cues19.

RESULTS
Platform information
Magnetic field gradients generated by magnetizing soft ferromagnetic micromagnets in close proximity to patterned cells allow for the remote generation of forces via coalescence of cell-internalized magnetic nanoparticles (Fig. 1). At the core of this platform is a micromagnetic substrate composed of (i) electroplated soft magnetic elements, (ii) a biocompatible, planarized resin and (iii) lithographically generated patterns of adhesive regions to precisely align magnetic nanoparticle–dosed cells with micromagnets. Magnetizing these micromagnetic elements with a permanent magnet generates arrayed magnetic potential minima

1Department of Bioengineering, University of California, Los Angeles (UCLA), Los Angeles, California, USA. 2Department of Electrical Engineering, UCLA, Los Angeles, California, USA. 3California NanoSystems Institute, UCLA, Los Angeles, California, USA. 4Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, California, USA. Correspondence should be addressed to D.D.C. (dicarlo@seas.ucla.edu).

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that rapidly and precisely localize nanoparticles inside cells\textsuperscript{20–22} (Fig. 1a), yielding highly consistent, force-generating nanoparticle ensembles over arrays of uniformly shaped cells (Fig. 1b). Cell patterning can generate 10,000–40,000 cell patterns per cm\textsuperscript{2}; we typically achieved a 50–75\% combined fill rate for single and multiple cells and a 20–25\% fill rate for single cells only. We additionally found that cells with internalized fluorescent nanoparticles can be sorted by fluorescence intensity with flow cytometry for improved uniformity of stimuli (Supplementary Fig. 1). The force on the cell cortex can be approximated through finite-element modeling and depends on several factors, including the volume of nanoparticle ensembles (Supplementary Fig. 1). Aspects of the fabrication can be tuned to achieve varying results: for example, modifying the thickness of the resin, and hence the distance of the cell from the elements, raises the force; the micromagnetic elements can be reduced in size and their localization can be varied for versatility in stimulus distribution (Fig. 1e).

Fabrication
We developed a process to fabricate functioning substrates with precise alignment between patterned cells and magnetically active elements (Supplementary Fig. 2). A nickel-iron alloy with a goal proportion of 70:30 (permalloy) was electroplated in a custom-designed setup\textsuperscript{26}. We extracted magnetic characteristics of the permalloy layer with a vibrating sample magnetometer (magnetization saturation, \(M_s = 1.13\) T) and verified the exact plating proportions of individual samples with energy-dispersive X-ray spectroscopy. We chose PSR resin—a biocompatible, low background-fluorescence resin\textsuperscript{27}—as the planar contacting substrate for cells. The resin was spun on over the protruding micromagnetic elements and processed to achieve high planarity. Thinner resin layers (with thickness 0.5–1.0 \(\mu\)m above the elements) had a mild topographical variation of 300 nm over a 10-\(\mu\)m length above the magnetic edges, whereas thicker resin varied less than 150 nm over a 15-\(\mu\)m length. Next we accomplished cell patterning using photolithography to allow alignment of cells with the micromagnets (Fig. 1d). Aspects of the fabrication can be tuned to achieve varying results: for example, modifying the thickness of the resin, and hence the distance of the cell from the elements, raises the force; the micromagnetic elements can be reduced in size and their localization can be varied for versatility in stimulus distribution (Fig. 1e).

Simulation and force verification
We simulated the magnetic response of individual elements in Comsol Multiphysics (Supplementary Fig. 3a) for measured fields generated from a 1-inch\(^3\) NdFeB magnet (K&J Magnetics; field measured by magnetometer). Because the size of the magnet is comparable to the substrate length, the field varies with position along the substrate relative to the permanent magnet. These measured fields primarily vary in the normal component of the magnetic field, \(B_n\), which decreases for elements nearer to the permanent-magnet center (Supplementary Fig. 3b). To determine force, we extracted magnetic field gradients either from finite-element simulation through curve fitting (Supplementary Fig. 3c) for various resin heights under the same stimulus or directly through the \(x\)-\(z\) variation of the field, for higher precision. Combined forces \(F\) on particle ensembles can be estimated according to

\[
F = \sum (m \nabla B)
\]
where \( m \) is the saturation moment of an individual nanoparticle, and the summation is over all localized nanoparticles. This equation is modified to

\[
F = \sum (k V(x,y) M_{\text{sat}} \mu_0^{-1} \nabla B(x,y,z))
\]

(2)

where \( V(x,y) \) is an estimate of the volume of the nanoparticle cluster at a position, \( \mu_0 \) is the permeability of free space, \( k \) is the packing volume fraction of nanoparticles and \( M_{\text{sat}} \) is the saturation magnetization of the nanoparticles, 0.11 T (nanomag-D plain, Micromod, conjugated to fluorophores in our laboratory).

In comparing wide-field fluorescence microscopy images with confocal images, we found that it is reasonable to assume a linear relationship between fluorescence intensity and thickness of the nanoparticle cluster: intensity of a pixel in the wide-field image was closely proportional to the number of nanoparticles in that square area. We then approximated force from wide-field images by summing forces at each pixel position, following equation (2). We estimated packing volume fraction \( k \) to be 0.6–0.8 based on electron micrographs\(^2\). We assessed the precision and accuracy of our generated magnetic field gradients through further finite-element simulation and experimental measurement. We assessed the precision of

Figure 2 | Effects of magnetic field gradient and nanoparticle loading on cell response. An array is shown of tiled cropped images of cells subject to increasing nanoparticle dose and magnetic field gradient. Cells were stained for actin (green), nanoparticles (blue) and DNA (cyan). The cells in the top right corner display ‘pull-in’ instability. The gradient varies from 2,500 T m\(^{-1}\) to 70,000 T m\(^{-1}\). The nanoparticle dose varies from 5 pg to 300 pg per cell. Scale bar, 10 \( \mu \)m. Note that the maximum intensity threshold for the actin channel was uniformly reduced so that filopodia are more visually apparent. Actin protrusions are not saturated and therefore retain a linear intensity mapping.

We assessed the precision and accuracy of our generated magnetic field gradients through further finite-element simulation and experimental measurement. We assessed the precision of

Figure 3 | Nanoparticle tension-dependent asymmetry in actin polymerization. (a) Single cells patterned using the indicated fibronectin shapes; colors are as in Figure 2. (b) Scatter plots with overlaid averages (error bars, s.d.) plotting the actin protrusion asymmetry for cells patterned by the fibronectin shapes in a. \( n \), number of cells per sample. Zero on the y axis corresponds to symmetric actin across the cell. Positive asymmetry indicates increased protrusive actin around the region of applied force as compared to the rest of the cell. The gray baseline in the samples is the average asymmetry as determined from control samples (excess nanoparticles under reduced magnetic field). (c) Percentage of cells at a given force level with actin asymmetry over 70%. Colored arrows denote ‘protrusion thresholds’, or the tension at which this percentage nears its maximum observed for the separate fibronectin shapes (green, square; teal, I; orange, X). (d) Comparison of cell yield tension (the lowest average tension at which nanoparticle clusters were observed to break through the cell membrane) on different adhesive patterns. Yield stress was estimated from yield tension and approximate nanoparticle thicknesses as obtained from confocal microscopy (1.5–2.3 \( \mu \)m, with an average of 1.8 \( \mu \)m). The average is used as our approximate thickness. Protrusion threshold is defined from c. Scale bar, 10 \( \mu \)m.
our finite-element–modeled gradients through simple perturbation tests of substrate parameters (Supplementary Fig. 3d). Inaccuracies in characterizing substrate parameters (cell position, magnetic element thickness and magnetic characteristics) yielded 3–7% variation from the expected force. We experimentally verified the accuracy of the modeled magnetic field gradients as a function of x–y–z positional variation from the micromagnet. We seeded magnetic beads ($M_s = 0.05$ T, MyOne, Invitrogen) in water onto substrates and imaged the trajectories of the beads in response to an applied field (Supplementary Video 2). To aid in the imaging of particle motion, we adjusted this applied field to around half the saturating field of our elements. We determined particle velocity as a function of position and calculated the Stokes drag at each distance to determine the force. Comparisons between forces generated by a simulated test sphere and forces determined from experimental trajectories showed excellent agreement (Supplementary Fig. 4) over the operating range of our substrates.

**Nanoparticle-induced tension generates filopodia asymmetry**

We first evaluated how our stimulus modifed the actin distribution within cells. We investigated cells patterned to square shapes using three distinct fibronectin patterns: [square], [I] and [X]. We simultaneously incubated arrays of cells on three separate substrates, with resin thicknesses of ~0.7, ~1.9 and ~4.3 μm above the micromagnetic elements, under magnetic stimulus for 4–5 h (depending on resin thickness), and we subsequently fixed and stained the cells for analysis. The three resin thicknesses, slight variations in cell position and varying quantities of nanoparticles per cell resulted in a range of applied forces. Qualitatively, as we increased particle-applied forces, filopodial protrusions appeared more frequently, emanating from the region to which we applied force (Fig. 2). We observed that at high stresses, nanoparticles occasionally extended and retracted within dynamic protrusions (Supplementary Video 1). Nanoparticles additionally began to generate clear deformations in the cell membrane (Supplementary Video 3). The largest forces created ‘pull-in’ instability, in which the nanoparticle clusters were pulled toward their magnetic minimum above the magnet, drawing the cell membrane along. When this occurred, actin response diverged (Fig. 2) as cells often expelled the nanoparticles, and actin stress fibers rebonded behind the nanoparticles. On rare occasions, dense actin projections emanated from the area of protrusion. We did not include data for cells at these extremes in our quantitative analysis as there was no longer a defined tension owing to destabilization of the cell membrane.

We analyzed large arrays of cells and collected quantitative data describing the effect of force magnitude on local actin protrusions (Fig. 3a). In addition, we estimated tensions and stresses at which the cell membrane yielded. We imaged magnetic substrates using fluorescence microscopy with the aid of a motorized stage, and we subsequently cropped, separated and analyzed the resulting images for average actin protrusion asymmetry (Fig. 3b and Supplementary Fig. 5). This metric is a normalized measure of the average actin protruding from the local area stimulated by nanoparticles (per unit length) compared with the average actin protruding from equivalent stress-fiber edge regions without adjacent nanoparticles (for X and square structures, all other edges; for I, only the opposite edge; Supplementary Fig. 4). As tension on the cell membrane increased, a larger fraction of cells displayed protrusion asymmetry (Fig. 3c and Supplementary Fig. 6); under a reduced holding force, we did not observe any

Figure 4 | Nanoparticle-mediated mechanical tension generates PAK-dependent filopodia. (a) Average intensity (in arbitrary units, a.u.) of filopodia around regions of induced tension plotted for three experiments for the indicated adhesion patterns. Low tension is 0–0.15 nN μm$^{-1}$ for square patterns and 0–0.3 nN μm$^{-1}$ for I and X patterns; high tension is 0.15 and 0.3–2.0 nN μm$^{-1}$, respectively. Near-yield tension is above 2 nN μm$^{-1}$ for all patterns. The images show typical cellular responses at moderately deforming tensions. The colors are as in Figure 2. (b) Plots (with legend as in a) from testing seven inhibitors of mechanotransductive proteins. Representative images for the indicated inhibitors are shown on the right. (c) z slices through two cells with different degrees of filopodial asymmetry displaying the activation of membrane-localized phospho-PAK (red). Arrows indicate a band of phospho-PAK (phosphorylated at serines 199 and 204) that unfolds regions of high deformation. (d,e) Cells stained for filopodial markers fascin (d; red, marked by arrow), myosin (myo)-X (e; red, localized to filopodia tips) and actin (e; green). Staining for DNA (cyan) and nanoparticles (blue) is the same in both images. Scale bars, 10 μm.
asymmetry. Scatter plots of actin protrusion asymmetry for three respective thicknesses are notably similar (Supplementary Fig. 6a). In addition, we observed no asymmetry in cells with nanoparticle quantities 2–4 times higher than those of cells used in force experiments and subjected to a magnetic field that was an order of magnitude lower (Fig. 3b and Supplementary Fig. 7).

Using these large data sets, we identified cellular effects of force. With increasing tension, neighboring actin stress fibers would often be disrupted at the position of force application (Supplementary Fig. 6). This effect was often localized in the z plane of the cell and was clearer in confocal images. We defined two quantitative thresholds: (i) the protrusion tension threshold at which an increased number of cells display asymmetric actin protrusions (Fig. 3c) and (ii) a yield tension threshold at which nanoparticle clusters destabilized the cell membrane (Fig. 3d). Our determined protrusion thresholds of 0.5–2.0 nN µm⁻¹ are consistent with the 1 nN of force generated by single pillars pulling on the cell exterior that polarize cellular biochemistry, whereas the yield thresholds are consistent with the stress applied by the leading edge of a lamellipodium during extension (1–2 nN µm⁻² to 10 nN µm⁻²). We found the protrusion threshold for cells adhered to the square shape to be lower than that for X and I shapes. Mechanically responsive proteins may already be recruited to the local environment near adhesive complexes interacting with the square fibronectin pattern, in contrast to the local protein environment for X and I shapes.

Filopodia are p21-activated kinase dependent

Protrusive actin structures at the site of force generation possessed biochemical characteristics of functional filopodia (Fig. 4). Protrusions stained positive for the markers fascin (Fig. 4d), myosin-X (Fig. 4e) and β integrin, which are commonly associated with active, leading edge–generated filopodia. We evaluated the production of filopodia as a function of cell-adhesive pattern for three ranges of force (low, high and near-yield tension) over multiple experiments with separate substrates and nanoparticle-loaded cells (Fig. 4a). Consistently with the previously described experiments, cells on I and X shapes generated a similar number of filopodia, which was lower than the number we observed in cells adhered to square patterns.

To characterize the origins of nanoparticle-induced filopodia, we systematically inhibited various mechanotransduction pathways. Several calcium-channel blockers—including GSMTx-4, streptomyccin and EGTA, which chelates calcium—and recently developed inhibitors of mechanotransductive proteins—ML141 and dasatinib (inhibitors of CDC42 and ABL1, respectively)—did not produce a noticeable effect on filopodia (data not shown). We tested the normalized filopodia-generation responses to seven other inhibitors of major mechanotransductive proteins (proteins shown in parentheses): CK869 (ARP 2/3 complex), wortmannin (multiple), PD98059 (extracellular signal–regulated kinases, MEK and ERK), PP2 (SRC), axitinib (multiple), NSC23766 (RAC) and IPA-3 (PAK); and we compared them to parallel uninhibited controls (Fig. 4b). Of the 12 inhibitors we tested, only two showed notable inhibition of the force-induced filopodia: wortmannin and IPA-3, both of which are known to affect aspects of PAK. Wortmannin-inhibited cells had fewer and shorter filopodia, whereas IPA-3 treatment eliminated most filopodia altogether (Fig. 4b), a finding consistent with research on PAK inhibition. About 45% of these treated cells exhibited non-apoptotic blebbing (Supplementary Fig. 8a). Blebbing cells exhibited similarly low intensities of filopodia to those of cells without blebbing, but we did not include these cells in our analysis.
We found that PAK localized to stress fibers and formed a distinct band along the regions of high deformation of the cellular cortex (Fig. 4c and Supplementary Video 4). Additionally, phospho-PAK localized to filopodia tips in cells grown on I and X patterns and was distributed throughout filopodia on square patterns and in regions close to focal adhesions on I and X patterns (Supplementary Fig. 8b). Overall, these data suggest that localized nanoparticle-mediated tensions lead to execution of a PAK-dependent biological program of filopodial generation that is similar in nature to filopodial generation at locations of cell adhesion and force application to the fibronectin substrate.

**Forces bias metaphase-plate orientation**

We used our setup to manipulate the organization of DNA and subsequent cell division during mitosis. The adhesive environment has been shown to direct the spindle axis, and subsequently the chromosomal organization and division axis of cells with extracellular force as the fundamental origin of this biasing. We observed that magnetic nanoparticle-mediated forces, when magnitudes were similar to those generated by actin on the cellular cortex (10–100 nN), caused effects similar to those from extracellular cortical forces, resulting in up to 90° shifts in the orientation of the mitotic spindle. We stimulated synchronized cells on magnetic substrates under three conditions (continuous maximal magnetic fields, high initial field subsequently modified to a lower holding value, or eventual 0 field), fixed the cells during mitosis and analyzed them. Cells in which maximal force was applied by nanoparticles showed cell-division axes and DNA orientation biased along the direction of force, whereas equivalently dosed cells in which magnetic stimulus was eventually reduced did not show bias (Fig. 5 and Supplementary Video 5). Inhibitor treatment with PP2, which has been shown to disrupt focal adhesion kinase and thus force sensing of retraction fibers, did not eliminate the observed biasing (Fig. 5c).

The biasing of cell-division axis and DNA orientation was more apparent for cells patterned on X and I shapes than for those on square fibronectin patterns. For both the X and I patterns, force was applied to the cell membrane in regions with no adhesive connections to the substrate, whereas in square patterns these forces overlapped with retraction fibers (Supplementary Fig. 9). Additional force generated from nanoparticles possibly competes with retraction fiber–induced force, reducing the overall change in local cortical tension for square patterns.

**DISCUSSION**

We demonstrated a technological platform for simultaneous, mechanical stimulation of thousands of cells, thereby addressing the biological noise inherent to single-cell activity and allowing researchers to obtain quantitative data on the cellular response to mechanical stimuli over a range of forces. We believe this approach has potential not just as a tool to study single-cell mechanical response but also as a means of cell control, potentially through modifying cell movement, division or differentiation. More generally, once approaches to release nanoparticles from endosomes are implemented (whether mechanically or chemically), the technique provides a platform to dynamically apply localized stimuli in cells. The annotated nanoparticles used in this study can be used to bind biomolecules: the bioconjugation of proteins, nucleic acids, small molecules or whole organelles should permit analyses of molecular localization and its importance in cell function.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Note:** Supplementary information is available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

P.T. and D.D.C. contributed to the fabrication design. D.D.C. and P.T. designed the integration of magnetic elements and single cells. P.T. developed the final fabrication and cell-patterning protocols. P.T. fabricated the micromagnetic slides and conducted the cell experiments. J.W.J. and P.T. discussed the finite-element simulation. P.T. designed the numerical analysis flow. P.T. and D.D.C. discussed and analyzed the numerical results. All authors wrote the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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1. Ingber, D.E. Tensegrity: the architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* 59, 575–599 (1997).
2. Orr, A.W., Helmeke, B.P., Blackman, B.R. & Schwartz, M.A. Mechanisms of actin-based propulsion: a direct measurement by micromanipulation. *Science* 257, 174–180 (1992).
3. Charra, G.T. & Horton, M.A. Single cell mechanotransduction and its modulation analyzed by atomic force microscopy. *Biophys. J.* 82, 2970–2981 (2002).
4. Prass, M., Jacobson, K., Mogilner, A. & Radmacher, M. Direct measurement of the lamellipodial protrusive force in a migrating cell. *J. Cell Biol.* 174, 767–772 (2006).
5. Dai, J. & Sheetz, M.P. Mechanical properties of neuronal growth cones measured by tether formation with laser optical tweezers. *Biophys. J.* 88, 988–996 (1995).
6. Wang, N., Butler, J.P. & Ingber, D.E. Mechanotransduction across the cell surface and through the cytoskeleton. *Science* 260, 1124–1127 (1993).
7. Laurent, F.M. et al. Assessment of mechanical properties of adherent living cells by bead micromanipulation: comparison of magnetic twisting cytometry vs optical tweezers. *J. Biomech. Eng.* 124, 408–421 (2002).
8. Huang, H. et al. Three-dimensional cellular deformation analysis with a two-photon magnetic manipulator workstation. *Biophys. J.* 82, 2211–2223 (2002).
9. Marcy, X., Prost, J., Carlier, M.-F. & Sykes, C. Forces generated during actin-based propulsion: a direct measurement by micromanipulation. *Proc. Natl. Acad. Sci. USA* 101, 5992–5997 (2004).
10. Hochmuth, R.M. Micropipette aspiration of living cells. *J. Biomech.* 33, 15–22 (2000).
11. Evans, E., Ritchie, K. & Merkel, R. Sensitive force technique to probe molecular adhesion and structural linkages at biological interfaces. *Biophys. J.* 74, 1580–1587 (2005).
14. Pelham, R.J. Jr. & Wang, Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. USA* **94**, 13661–13665 (1997).

15. Banes, A.J. et al. Mechanoreception at the cellular level: the detection, interpretation, and diversity of responses to mechanical signals. *Biochem. Cell Biol.* **73**, 349–365 (1995).

16. Sniadecki, N.J. et al. Magnetic microposts as an approach to apply forces to living cells. *Proc. Natl. Acad. Sci. USA* **104**, 9453–9458 (2007).

17. Hui, E.E. & Bhatia, S.N. Micromechanical control of cell-cell interactions. *Proc. Natl. Acad. Sci. USA* **104**, 14553–14558 (2007).

18. Tanase, M. et al. Assembly of multicellular constructs and microarrays of cells using magnetic nanowires. *Lab Chip* **5**, 598–605 (2005).

19. Fink, J. et al. External forces control mitotic spindle positioning. *Nat. Cell Biol.* **13**, 246–250 (2011).

20. Gao, J. et al. Intracellular spatial control of fluorescent magnetic nanoparticles. *Nat. Nanotechnol.* **3**, 36–40 (2008).

21. Huang, H. et al. Remote control of ion channels and neurons through magnetic-field heating of nanoparticles. *Nat. Nanotechnol.* **5**, 602–606 (2010).

22. Delorme-Walker, V.D. et al. Pak1 regulates focal adhesion strength, myosin IIA distribution, and actin dynamics to optimize cell migration. *J. Cell Biol.* **193**, 1289–1303 (2011).

23. Van den Broeke, C. et al. Alphaherpesvirus US3-mediated reorganization of the actin cytoskeleton is mediated by group A p21-activated kinases. *Proc. Natl. Acad. Sci. USA* **106**, 8707–8712 (2009).

24. Théry, M. et al. The extracellular matrix guides the orientation of the cell division axis. *Nat. Cell Biol.* **7**, 947–953 (2005).

25. Théry, M., Jiménez-Dalmonori, A., Racine, V., Bornens, M. & Jülicher, F. Experimental and theoretical study of mitotic spindle orientation. *Nature* **447**, 493–496 (2007).
ONLINE METHODS

Magnetic fluorescent nanoparticle preparation. A proportion (4:2.5) of suspended dextran-magnetic nanoparticles (nanomag-D, plain –OH, Micromod), 10 M NaOH and epichlorohydrin was reacted for 24 h under darkness40. Ammonium hydroxide was then added in excess (in the same proportion as the initial nanoparticle colloid) and reacted for another 24 h in darkness, and the final suspension was dialyzed exhaustively in DI water. The suspension was separated in multiple cycles with a permanent magnet and finally concentrated to its approximate initial concentration in pH 8.3 bicarbonate buffer. Aminated magnetic fluorescent nanoparticles were subsequently reacted with 10 µg ml⁻¹ Alexa Fluor 647 succinimidyl ester or Alexa Fluor 568 succinimidyl ester (Invitrogen). Nanoparticles were tested for brightness and internalization and were reacted with more fluorophore as needed (typically under 20 µg ml⁻¹ total fluorophore). The suspension was again separated in multiple cycles by permanent magnet, in addition to gentle heating (55 °C) to stabilize the final colloid, before finally being suspended in PBS and stored at 4 °C.

Fabrication of micromagnetic slides. Polished borosilicate glass (Tech Gophers) or glass slides (Fisher) were cleaned in heated piranha solution for 30 min, washed with DI water and subsequently acetone, methanol and isopropanol, before finally being subjected to oxygen plasma cleaning in a barrel asher. A 30-nm-Th, 250-nm-Cu and 30-nm-Ti seed layer was then evaporated onto the substrate. KMPr photoresist was spun and processed according to specification to form the electroplating mold for nickel-iron alloy. Titanium was etched in 1% HF, and Ni₃Fe₂ (goal: 70:30) was electroplated in a custom plating setup (bath: 250 g L⁻¹ NiO₄·7H₂O, 5 g L⁻¹ FeSO4·7H2O, 25 g L⁻¹ boric acid, 1 g L⁻¹ saccharin and 1 g L⁻¹ sodium lauryl sulfate, pH 3.0) with a goal current density of 3 mA cm⁻² and a thickness of approximately 10 µm. Photoresist was stripped in Aleg 355, and the seed layer was etched in copper etchant (1% HF, 5% acetic acid, 15% H₂O₂) and titanium etchant (1% HF). The metal layer was then passivated by deposition of 100 nm PECVD Si₃N₄. PSR photoresist (a gift from M. Bachman) was processed to optimize substrate planarity. A mix of PSR-10 and PSR-50 was spun with an acceleration of 500 r.p.m. s⁻¹ up to an r.p.m. for obtaining desired substrate thickness (typically 2,500–3,600 r.p.m.). The substrate was baked for 1 min at 65 °C before being ramped to 95 °C within 3 min and was then baked at 95 °C for 25 min before the hotplate was turned off. The resist was subsequently exposed, post-exposure baked for 1 min at 65 °C and then 3 min at 95 °C and finally cured under nitrogen at 120 °C for 10 min. Cells were patterned using a lithographic approach and AZ5214E was prebaked, exposed and developed to form the protein pattern.

Cell preparation. HeLa cells were incubated with varying concentrations of nanoparticles (dependent on nanoparticle fabrication yield). In general, for optimally processed nanoparticles, cells were incubated in 50 µg ml⁻¹ of nanoparticles in DMEM at 37 °C for 20–60 min (the final required time is dependent on the desired dosage of nanoparticles in cells—this can be approximated by eye under a microscope) before being washed aggressively in PBS and rewarshed in DMEM. Cells were allowed to recover after washing for a minimum of 2 h before use (longer recovery times yield cleaner substrates). The average number of nanoparticles per cell (that is, the dosage) depends on nanoparticle concentration, cell incubation time and nanoparticle surface chemistry (Fig. 5 and Supplementary Fig. 1).

Cell patterning protocol. To prepare substrates for cellular adhesion, 40 µg ml⁻¹ fibronectin and 25 µg ml⁻¹ fibrinogen–Alexa Fluor 568 solutions were pipetted onto the surface and incubated for 2 h in a Petri dish. The surface was washed aggressively with PBS and allowed to settle in PBS for 5 min before the protein mask was stripped in ethanol for 1 min with two 5-s ultrasonic pulses. The substrate was again washed with PBS before being incubated in 2% pluronic F-127 (Sigma) for 50 min. The substrate was finally washed three times in PBS and incubated in warm DMEM in a sterile Petri dish. Prepared cells were trypsinized, pelleted, resuspended in DMEM and then pipetted above the substrate to achieve a goal of 10 cells per 10,000 µm². Cell adhesion was checked at 10-min intervals, excess cells were washed in DMEM and the cells were allowed to settle on the substrate for a minimum of 2 h before subsequent experimentation.

Flow cytometry of nanoparticle-internalized cells. Cells were sorted in a BD Aria II FACS sorter with a 100-µm nozzle at 20 p.s.i. Two separate cells populations, after overnight incubation following nanoparticle dosing, were either sorted by FACS or not sorted. We seeded cells from these experiments onto separate substrates and coalesced nanoparticles by permanent magnet for 2.5 h. Samples were then imaged with fluorescence microscopy for quantification of nanoparticled nanoparticles.

Actin asymmetry experiments. An NdFeB rare earth magnet (1 inch x 1 inch x 1 inch, K&J Magnetics) was applied to the bottom of a cell-seeded, magnetic substrate–containing Petri dish, and the dish was incubated at 37 °C. The cells were allowed to stabilize to their final state over 4–5 h depending on the combined magnetic element and resin thickness. Control samples used thick substrates (4–5.5 µm) with cells overloaded with nanoparticles that were localized under high field for 2 h but had the magnet removed so that the incident field was approximately 0.01 T or 0.025 T for 2 h. Upon completion, the substrates were quickly washed in warm 3% formaldehyde and allowed to incubate in solution at 37 °C. After 10 min, the magnet was removed, and the substrate was washed three times in PBS. Cells were permeabilized in 0.5% Triton X-100 (Sigma), washed with PBS and incubated in three AU phallolidin–Alexa Fluor 488 conjugate for 15 min and washed again in PBS. Cell slides were lastly cover glass mounted in Vectashield with DAPI medium (Vector Lab) before being sealed with nail polish.

Antibodies and inhibitors. Antibodies to myosin-X (1:1,000, Novus, cat. no 22430002), β integrin (2 µg ml⁻¹, Millipore, clone HM beta 1.1) and phospho-PAK (Ser199/204) (1:400, Millipore, cat. no. 09-258) were incubated with 3% formaldehyde–fixed and Triton-X–permeabilized cells overnight at 4 °C. Fascin antibody (1:100, Santa Cruz Biotechnologies, clone 55K2) was incubated with methanol-fixed cells for 1 h at room temperature. We then incubated all samples with corresponding secondary antibody (Alexa Fluor 568, Invitrogen, 1:500) for 45 min before mounting samples in either Vectashield or SlowFade with DAPI (Invitrogen). For phospho-PAK staining, saponin (0.1%) was used instead of Triton X-100 for permeabilization.
Streptomycin (1 mM, 2 h, Sigma), GSMTx-4 (25 µM, 30 min, Sigma), EGTA (5 mM, 2 h, Sigma in Ca-free medium), CK869 (ref. 42) (30 µM, 1 h, Sigma), PP2 (20 µM, 1 h, Sigma), wortmannin (750 nM, 1 h and again 10 min, Sigma), ML141 (10 µM, 1 h, Tocris), NSC23766 (100 µM, 1 h, Tocris), axitinib (10 nM, 1 h, Tocris), PD98059 (50 µM, 1 h, Sigma), dasatinib (200 nM, 1 h, LC Labs) and IPA-3 (ref. 43) (30 µM, 30 min, Sigma) were added to high-glucose DMEM, 10% serum and 1% penstrep medium at notated times before magnet application. We then excited these samples by magnet for 1–1.25 h (varying with substrate thickness) before we fixed, stained and analyzed cells as in previous actin quantification experiments. Our inhibition experiments consisted of simultaneously running mini-groups of samples, typically 2 or 3 samples with added inhibitors alongside a single standard sample in normal culture medium (with or without DMSO) as the control for the group. We calculated inhibitory data as a percentage of this control response.

Cell-division experiments. Before nanoparticle internalization, we synchronized HeLa cells by double thymidine block and allowed these to rest for 2 h before exposing them to nanoparticles. The protocol then followed actin asymmetry experiments identically except that approximately 1 h before estimated onset of mitosis, external magnets were either removed or positioned away from the sample to achieve an approximately 0.025-T incident field so as to modulate the asymmetric force on the cells. Cells were inspected under the microscope for rounding and then were fixed and stained when a sufficient number of cells were rounded and dividing. Mitotic spindle angle of dividing cells was estimated as perpendicular to the DNA orientation in pre-anaphase cells and perpendicular to the bisecting angle of divided post-anaphase cells.

Imaging. Wide-field fluorescence images were captured using a Nikon inverted fluorescence microscope with a 20× objective lens on a Photometrics Coolsnap HQ2. Stitched images were captured using NIS-Elements and subsequently extracted and cropped for analysis. High-speed images were captured with a Phantom Cinestream v.711 camera (Vision Research) with a 40× objective running at 2,000 images per s. Confocal images were captured using a Leica SP2 microscope. Live-cell imaging was conducted in a fluorescence microscope–incubator setup. Substrates were inverted over a plastic spacer and clamped in place. The magnet was suspended above the substrate and medium, and the sample was subsequently imaged.

40. Pande, A.N., Kohler, R.H., Aikawa, E., Weissleder, R. & Jaffer, F.A. Detection of macrophage activity in atherosclerosis in vivo using multichannel, high-resolution laser scanning fluorescence microscopy. J. Biomed. Opt. 11, 021009 (2006).
41. Guillou, H. et al. Lamellipodia nucleation by filopodia depends on integrin occupancy and downstream Rac1 signaling. Exp. Cell Res. 314, 478–488 (2008).
42. Nolen, B.J. et al. Characterization of two classes of small molecule inhibitors of Arp2/3 complex. Nature 460, 1031–1034 (2009).
43. Deacon, S.W. et al. An isoform-selective, small-molecule inhibitor targets the autoregulatory mechanism of p21-activated kinase. Chem. Biol. 15, 322–331 (2008).