We demonstrate substantial differences in ‘adhesive signature’ between human pluripotent stem cells (hPSCs), partially reprogrammed cells, somatic cells and hPSC-derived differentiated progeny. We exploited these differential adhesion strengths to rapidly (over ~10 min) and efficiently isolate fully reprogrammed induced hPSCs (hiPSCs) as intact colonies from heterogeneous reprogramming cultures and from differentiated progeny using microfluidics. hiPSCs were isolated label free, enriched to 95%–99% purity with >80% survival, and had normal transcriptional profiles, differentiation potential and karyotypes. We also applied this strategy to isolate hPSCs (hiPSCs and human embryonic stem cells) during routine culture and show that it may be extended to isolate hPSC-derived lineage-specific stem cells or differentiated cells. Generation of hiPSCs from somatic cells is a promising strategy to produce autologous cells for regenerative therapies and novel models of human development and disease. However, the reprogramming process is inefficient (0.001%–2% reprogrammed cells), and hiPSC cultures are often heterogeneous because of the presence of undifferentiated stem cells, parental and partially reprogrammed cells and differentiated derivatives, thereby introducing variability, potential immunogenicity and problems in directed differentiation. hiPSC survival and ‘stemness’ require compact colonies with E-cadherin–mediated cell-cell adhesion. Current methods for maintenance of hiPSC cultures rely on manual isolation, either alone or in combination with enzymatic dissociation. Such methods are time intensive, require skilled labor and are dependent on morphologic recognition of undifferentiated cells. Although many reagents have been developed for enzymatic passaging, such methods are not selective for hPSCs, and unwanted cells are often transferred during passaging. Furthermore, enzymatic methods have been reported to be associated with karyotypic abnormalities when compared to manual passaging and require reaggregation of dissociated hPSCs for improved survival. This method requires single-cell dissociation of hPSCs, which induces cell death, and replated cells fail to form compact colonies (Supplementary Fig. 1).

There remains a need to develop unbiased, high-throughput technologies that can efficiently separate colonies of hiPSCs from nonreprogrammed and partially reprogrammed cells, feeder cells or differentiated cells. In this study, we demonstrate a unique ‘adhesive signature’ for hiPSCs that is a multifactorial function of extracellular matrix (ECM)-bound integrins, assembly of focal adhesions and the resulting cell-ECM adhesion strength. We present a platform technology that exploits differences in the adhesion strength among nonreprogrammed and partially reprogrammed cells, hiPSCs and differentiated cells to selectively isolate hiPSCs using microfluidics.

RESULTS
Changes in adhesive signature with reprogramming
During reprogramming to pluripotency, cells undergo changes in morphology resulting in an epithelial phenotype indicative of the pluripotent state (Fig. 1a). IMR90 and dermal fibroblasts, common somatic cell sources for reprogramming, exhibited an elongated (‘spread’) morphology and lack of pluripotency markers (Supplementary Fig. 2a–c) as compared to hiPSCs, which existed as tightly packed colonies (Supplementary Fig. 2d,e). Typically we observed that <1% of fibroblasts were converted to fully reprogrammed hiPSCs as compared to hiPSCs, which existed as tightly packed colonies (Supplementary Fig. 2d,e). Residual nonreprogrammed and partially reprogrammed cells exhibited a less elongated morphology than that of parental fibroblasts and expressed some, but not all, pluripotency markers (Supplementary Fig. 2e).

The adhesive strength of a cell to its ECM is dependent on ECM-ligated integrins and their association to cytoskeletal elements. Using flow cytometry and adhesion-inhibition studies (Supplementary Fig. 3), we determined that whereas parental fibroblasts expressed predominantly α5β1-integrin, hiPSCs expressed high levels of α6β1-integrin, regardless of whether the cells were cultured on fibronectin, laminin or Matrigel.
These results are consistent with those of human embryonic stem cell (hESC) studies. Fibroblasts possessed actin stress fibers, and vinculin and talin were enriched at focal adhesions (Supplementary Figs. 4 and 5). In contrast, hiPSCs exhibited substantially fewer actin fibers, with diffuse vinculin and talin throughout the cytoplasm or localized to cell-cell junctions. Nonpluripotent cells in reprogramming cultures showed mixed regions of spread cells with well-defined focal adhesions and round cells without distinct focal adhesions (Supplementary Fig. 4). We therefore hypothesized that alterations in the adhesive signature related to integrin binding and cytoskeletal components accompany reprogramming to induced pluripotency and differentiation of hiPSCs.

We evaluated the steady-state cell-ECM adhesion strength for hiPSCs and IMR90 cells using a spinning-disk device (Supplementary Fig. 6a). We observed that adhesion strength to fibronectin for hiPSCs was one-seventh that for parental fibroblasts (Fig. 1b). Analyses among fibroblastic parental and feeder cells, hESCs and hiPSCs revealed significantly lower adhesion strength to fibronectin, laminin and Matrigel for hPSCs than for fibroblasts (P < 0.02; Fig. 1b and Supplementary Fig. 6b), indicating that the adhesive properties of cells shift during reprogramming. These results were independent of passage number, underlying matrix, parental fibroblast source and colony size (Supplementary Figs. 6c and 7). The differences in adhesive strength correlated to more focal adhesions in parental cells than in hiPSCs. Nonreprogrammed and partially reprogrammed cells that expressed some but not all pluripotency markers (for example, OCT4 "SSEA4") exhibited higher adhesion strength than hiPSCs but lower adhesion strength than parental cells (Fig. 1c).

Distinct adhesive properties of differentiated cells

We next determined the adhesive signature of hiPSCs undergoing spontaneous or directed differentiation (Fig. 1d,e). We detected significant increases in the adhesion strength to the ECM of spontaneously differentiated cultures of hiPSCs (~10% TRA-1-60+) as compared to undifferentiated hiPSCs (Fig. 1f, P < 0.006); we obtained similar results in hESCs. Cells in spontaneously differentiating cultures of hiPSCs displayed actin stress fibers and localized talin and vinculin to focal adhesions (Fig. 1g), which we did not observe in undifferentiated colonies. Differences in adhesion strength between undifferentiated and differentiated cells were independent of the levels of spontaneous differentiation (Supplementary Figs. 5 and 8), which we did not observe in undifferentiated colonies. Differences in adhesion strength between undifferentiated and differentiated cells were independent of the levels of spontaneous differentiation (Fig. 1g).

During directed differentiation, we observed early-stage multipotent neural stem cells (neural rosettes) with a radial pattern of epithelial morphology (Fig. 1d), which stained for nestin (Fig. 1e) and Musashi (Supplementary Fig. 9a), distinct from hiPSCs. The adhesion strength of these cells was comparable to that of hiPSCs (Fig. 1h) but significantly lower than that of fibroblast-like cells (P < 0.05). We manually isolated rosettes and differentiated them to neural progenitors and neurons (Supplementary Fig. 9b). Neural progenitors exhibited adhesion strength comparable to that of neurons but 50% lower than that of hiPSCs (Fig. 1h,i).
and about 85% lower than that of spontaneously differentiated fibroblastic cells (Fig. 1g), independent of hPSC type and matrix (Fig. 1i). These analyses demonstrate that hPSCs, progenitors and differentiated cells exhibit distinct adhesive signatures.

**Hydrodynamic isolation of hPSCs**

We exploited the unique adhesive signature of hiPSCs to isolate undifferentiated hPSCs from a heterogeneous cell population. We used adhesive force–based separation via a simple microfluidic system, a label-free technique that requires minimal cell processing. We termed this technology μSHEAR (micro-stem cell high-efficiency adhesion-based recovery). We fabricated μSHEAR devices for a range of culture surface areas (Fig. 2a and Supplementary Fig. 10a). Cells remained viable in the device and retained their distinct morphologies, and hiPSCs remained undifferentiated (Supplementary Fig. 10b,c). The application of laminar flow generated fluid shear stresses on adherent cells in the device. hiPSC colonies detached at a shear stress of 85–125 dynes cm⁻² within 4 min of fluid-flow application and were completely detached in 10–14 min (Fig. 2b,c) irrespective of the underlying ECM (Supplementary Fig. 10d,e), whereas fibroblasts remained attached. To quantify the efficiency of hiPSC purification, we incubated recovered cells with StainAlive Dylight 488–conjugated TRA-1-60 antibody (Stemgent) to stain for live hiPSCs and with CellTracker Red dye (CMPTX; Life Technologies). Flow cytometry analysis of hiPSC (IMR90)

**Figure 2** | Adhesion strength–based isolation of pluripotent stem cells in microfluidic devices. (a) Schematic of μSHEAR device cross-section and scale-up. (b,c) Selective isolation of hiPSCs at a shear stress of 85–125 dynes cm⁻² when cocultured with IMR90 cells at low (b) and high (c) density. The white arrowheads indicate a hiPSC colony that is detached by flow. The red arrowheads indicate IMR90 fibroblasts. Scale bars, 200 μm. (d) Flow cytometry plots showing detached hiPSCs (TRA-1-60+CMPTX⁺) and IMR90 cells (TRA-1-60−CMPTX−) at the indicated shear stresses. After μSHEAR, residual cells in devices were trypsinized and analyzed. (e,f) Enrichment of hiPSCs and hESCs isolated at 85–125 dynes cm⁻² from a coculture with IMR90 and mouse embryonic fibroblast cells, respectively. Graphs show mean ± s.d. (⁎ P < 0.05, n = 3). (g) Detached hiPSC colonies readded to Matrigel and immunostained for the indicated markers. Scale bars, 50 μm. (h) Karyotype analysis of hiPSCs isolated twice using μSHEAR, with passages 7–8 d apart. (i) Embryoid bodies generated from μSHEAR-isolated hiPSCs immunostained with markers for the three germ layers (21 d). Scale bars, 50 μm, except the phase-contrast embryo bodies (left; scale bar, 200 μm).
the recovered cells revealed significant ($P < 0.05$) enrichment of hiPSCs when detached at 85–125 dynes cm$^{-2}$ with up to 99% purity (Fig. 2d) as compared to the initial purity of 39% hiPSCs. Exposure to higher fluid forces (250–350 dynes cm$^{-2}$) resulted in contamination with IMR90 cells (18%), whereas we observed <1% fibroblast contamination in cultures exposed to 85–125 dynes cm$^{-2}$. When we increased the fluid force to 750–850 dynes cm$^{-2}$, we observed high proportions of IMR90 cells in the detached populations, similar to those in trypsinized samples under no-flow conditions (Fig. 2d). The μSHEAR isolation efficiency was independent of hiPSC purity in the initial coculture (Fig. 2e,f and Supplementary Fig. 11a). Less than 3% of residual cells in devices after fluid detachment were hiPSCs, indicating high recovery yield of hiPSCs by μSHEAR (Fig. 2e,f). Similar results were observed with hESCs cultured on mouse embryonic fibroblast (MEF) feeders (Fig. 2e and Supplementary Fig. 10f). We observed similar enrichment with varying levels of hiPSCs in a fibroblast-hiPSC coculture (baseline hiPSC purity, 1%–70%). Finally, we

![Figure 3](image_url)

**Figure 3** | Adhesion strength–based isolation of hiPSCs from a heterogeneous reprogramming culture. (a) Heterogeneous reprogramming culture seeded into a μSHEAR device and subjected to a shear stress of 100 dynes cm$^{-2}$ for 5 min. The red arrowhead indicates a hiPSC colony that is detached by flow. The white arrowheads indicate nonreprogrammed or partially reprogrammed cells. Scale bar, 200 μm. (b) Center, flow cytometry plot showing detached hiPSCs (TRA-1-60$^+$CMPTX$^+$) and nonreprogrammed or partially reprogrammed cells (TRA-1-60$^+$CMPTX$^-$) at a shear stress of 100 dynes cm$^{-2}$. Right, analysis of residual cells in the device after μSHEAR. Left, analysis of an unpurified reprogramming culture in devices with baseline 0.65% hiPSC purity. (c–h) μSHEAR-isolated hiPSCs and residual cells from the devices replated on Matrigel and stained for the indicated markers. The white arrowheads indicate partially reprogrammed cells expressing pluripotency markers. Scale bars, 50 μm. (i) Representative hematoxylin-and-eosin–stained sections from a formalin-fixed teratoma produced from μSHEAR-isolated hiPSCs. μSHEAR-isolated cells formed differentiated tissues representing all three embryonic germ layers. Scale bars: 100 μm (top row), 25 μm (bottom row).
could also selectively enrich hiPSCs after the cells were allowed to proliferate with parental cells for 5–7 d in microfluidic devices (Supplementary Fig. 11b,c and Supplementary Video 1).

We used µSHEAR to efficiently separate hiPSCs from other parental cell types: specifically, from peripheral blood mononuclear cells (PBMCs) in blood25. Because PBMCs are loosely adherent (Supplementary Fig. 12a,b), we first exposed the reprogrammed culture to a shear stress of 10 dynes cm\(^{-2}\) to remove PBMCs, which were removed from the devices within 1 min of exposure to flow (Supplementary Fig. 12c). Thereafter, we collected hiPSCs as

![Image](https://example.com/image)

**Figure 4** | Adhesion strength–based enrichment of hiPSCs from differentiating cultures. (a,b) Flow cytometry histograms (Alexa Fluor 488–TRA-1-60) showing purification (a) and survival efficiencies (b) of hiPSCs processed as indicated. (c) hiPSCs (TRA-1-60–CMPTX\(^+\)) and spontaneously differentiated cells (TRA-1-60–CMPTX\(^−\)) detached as indicated at passages 1 and 10. (d) Enrichment efficiency of hiPSCs upon repeated passing with the indicated methods. P0 cells for all plots were from the same batch with 90% TRA-1-60\(^+\) cells; recovered cultures were propagated for 5–6 d. (e,f) Cell survival (e) and growth curves (f) of cells on Matrigel after passing as indicated, D. day. (g) Immunostaining for pluripotency markers of µSHEAR-isolated hiPSCs cultured on Matrigel across ten passages. Scale bars, 200 \(\mu\)m. (h) Fold change in expression of stem cell–related (left) and differentiation (right) genes in hiPSCs at P10 relative to at P0. D1–D3 represent triplicate runs of µSHEAR devices, and M1–M3 represent manual passing triplicates. (i) Relative expression of stem cell–related genes in hiPSCs isolated manually or using µSHEAR for ten passages. Magenta lines indicate a twofold change in gene expression. (j) Karyotype analysis of µSHEAR-passaged hiPSCs at P10. Error bars, s.d. (*\(P < 0.05\), n = 3).
colonies with ~99% purity by increasing the flow (Supplementary Fig. 12d and Supplementary Video 2).

hiPSCs recovered by µSHEAR initially adhered as small colonies (Fig. 2g) with the ability to self-renew without any signs of differentiation (Supplementary Fig. 13). The isolated colonies retained their pluripotent phenotype (Fig. 2g and Supplementary Fig. 13) and exhibited no chromosomal abnormalities after two rounds of purification with passages 7–8 d apart (Fig. 2h). µSHEAR-isolated hiPSC colonies readily generated embryoid bodies and differentiated into mesoderm, ectoderm and endoderm derivatives (Fig. 2i).

Isolation of hiPSCs from reprogramming cultures

We anticipated that adhesive signature differences could be exploited to selectively isolate hiPSCs from partially reprogrammed cultures. Using µSHEAR, we isolated hiPSC colonies (94% ± 3.6% purity) at 100 dynes cm⁻² without detachment of nonreprogrammed and partially reprogrammed cells (Fig. 3a,b and Supplementary Fig. 14a,b). We observed only 0.05% residual hiPSCs, whereas non-hiPSCs constituted 99.9% of the culture remaining in the µSHEAR device (Fig. 3b and Supplementary Fig. 14c). Isolated hiPSCs expressed TRA-1-60, TRA-1-81, OCT4, SSEA4, GDF3, hTERT and NANOG, indicating that they were fully reprogrammed. We also analyzed the methylation patterns of endogenous OCT4 (POUSF1), NANOG and SOX2 genes. µSHEAR-isolated hiPSCs displayed unmethylated OCT4, SOX2 and NANOG, similarly to hiPSCs under standard culture conditions and to unmethylated genomic DNA controls (Supplementary Fig. 14d). Finally, µSHEAR-isolated hiPSCs formed teratomas when implanted into immunodeficient mice (Fig. 3i and Supplementary Fig. 15). These studies demonstrate that fully reprogrammed, bona fide hiPSCs can be selectively isolated from residual parental fibroblasts and from partially reprogrammed cells using µSHEAR.

Isolation of hiPSCs from differentiated cells

We exploited the adhesive signature of undifferentiated hiPSCs to effectively separate them from differentiated progeny (Fig. 1f). Spontaneously differentiating hiPSC cultures with varying levels of differentiation were dissociated and cultured overnight in µSHEAR devices with hiPSCs (Supplementary Fig. 16a). We could isolate hiPSCs as intact epithelial colonies before detaching differentiated fibroblast-like cells with >97% purity and yield irrespective of the levels of spontaneous differentiation (6%–70% TRA-1-60⁻), and we observed similar results with hESCs (Supplementary Fig. 16b–d). We did not achieve selective purification with commonly used enzymatic agents (Supplementary Fig. 16b).

hPSC isolation with µSHEAR and TrypLE required 2–3 h to achieve comparable efficiency. Using µSHEAR, we isolated hiPSCs on average in ~30 min. µSHEAR-purified hPSCs expressed OCT4, hTERT and GDF3 but not the corresponding genes. We also confirmed the expression of differentiation and lineage-specific genes was equivalent or downregulated for both µSHEAR- and manually passaged hiPSCs as compared to starting P0 cells (Fig. 4h). Hierarchical clustering and scatter-plot analyses of gene expression at P10 indicated that µSHEAR-purified hiPSCs exhibited a high degree of similarity to manually passaged cells (Fig. 4i, j). µSHEAR-purified hiPSCs exhibited no chromosomal abnormalities at P10 (Fig. 4j) and showed the same methylation status of OCT4, SOX2 and NANOG as manually passaged hiPSCs (Supplementary Fig. 18d).

We applied µSHEAR to isolate terminally differentiated cells. Because their adhesion strength is lower than that of hiPSCs, neurons were detached at 60 dynes cm⁻², whereas hiPSCs remained adherent to the substrate at this shear stress level. Isolated neurons exhibited excellent viability, neurite growth (Supplementary Fig. 19) and expression of MAP2 and β-III tubulin. Similarly, we successfully isolated hiPSC-derived cardiomyocytes (Supplementary Video 3) from hiPSCs with >95% purity (Supplementary Fig. 20a,b). Recovered hiPSC adhered as colonies, whereas the residual cardiomyocytes expressed α-smooth muscle actin and exhibited spontaneous contractile activity (Supplementary Fig. 20c,d and Supplementary Video 4).

DISCUSSION

High-throughput microfluidic devices are being adapted in routine cell culture and offer advantages over conventional hydrodynamic sorting, including laminar flow with only a millionth of the buffer volume and the ability to recover detached cells. We demonstrate that differences in adhesion strength can be exploited to purify undifferentiated hPSCs from other cell types in a facile, efficient and label-free manner, yielding higher hPSC survival than conventional methods provide. Our µSHEAR methodology allows the application of a wide range of shear forces with small working volumes and precise magnitudes of shear force. We also tested the high-throughput potential of µSHEAR across culture areas of 0.5–9 cm² and found 95%–99% enrichment efficiency of hiPSCs, demonstrating that the technology is potentially scalable.
Cells are loaded in <30 s, and no time is spent precleaning the cultures. Self-contained disposable microfluidic devices ensure sterility, and cell recovery takes ~5 min, similar to the time required for a routine centrifugation step after enzymatic dissociation. In addition, the microfluidic strategy provides for direct visualization of detachment process of stem cell colonies; a gradient of shear forces could thus be used to serially isolate individual colonies from the same chamber, which could not be achieved with any bulk passing methods. μSHEAR will facilitate the integration of cell isolation procedures, such as separating completely reprogrammed hiPSCs from partially reprogrammed cells, with in-line biochemical, genomic, proteomic and metabolomic analyses.

**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**

A.S. and S.S. conducted all adhesion and microfluidic studies, collected data and performed data analysis. S.S., A.S. and H.L. developed microfluidic methods. J.M.C. and S.L.S. established and provided the IMR90-derived hiPSC cells and neural stem cells. T.L., W.C. and J.F. developed and provided micropatterns. M.T.C. and A.S. conducted microarray and epigenetic analysis. A.J.G. and T.C.M. developed the concept, and together with A.S. contributed to the planning and design of the project. A.S., S.S., T.C.M., and A.J.G. wrote the manuscript, and all authors discussed the results and commented on the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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hiPSC reprogramming and hiPSC culture. hiPSCs (IMR90) were derived and validated by Aruna Biomedical using the viPSP Vector Kit (Thermo Scientific/Open Biosystems) consisting of six lentiviral vectors encoding OCT4, NANOG, SOX2, LIN28, KLF4 and c-MYC (MYC) driven by the EF1α promoter. IMR90 human fetal lung fibroblasts (female, ATCC) were transduced (MOI of 10 for each vector) for reprogramming per the manufacturer's protocol. Transduced fibroblasts were seeded onto inactivated MEFs or Matrigel to form colonies, and emerging hiPSC colonies were manually passaged by mechanical dissociation on day 30. hiPSCs demonstrated well-defined borders, high nuclear-to-cytoplasmic ratio, prominent nucleoli, alkaline phosphatase activity, positive expression of cell-surface marker SSEA4, embryoid body formation and teratoma formation. To transition hiPSCs to a feeder-free culture system, we manually passaged colonies by mechanical dissociation onto Matrigel (1:100 dilution; BD Biosciences) in mTeSR1 medium (Stemcell Technologies). hiPSCs used in the study were between passages 26 and 48, routinely cultured as feeder-free undifferentiated hiPSC colonies in mTeSR1 medium on Matrigel and enzymatically passaged with dispase (1 mg mL⁻¹) and scraped. To maintain an undifferentiated state in hiPSCs, we exchanged the mTeSR1 medium daily. For μSHEAR experiments, reprogramming was performed on Matrigel, and the entire culture was introduced into the devices after 27–30 d. To obtain spontaneously differentiated cultures of hiPSCs, we exchanged mTeSR1 medium every second day and did not clean the cultures.

Human dermal fibroblast–derived hiPSCs (11b, healthy male donor) were obtained from Harvard Stem Cell Institute and cultured as above. Human peripheral blood mononuclear cell–derived hiPSCs were a gift from J. Wu (Stanford University) and were derived using Sendai virus. hESCs (H1 and H7) used in the study were at passage 35 (H1, WiCell) or passage 54 (H7, WiCell) and cultured in mTeSR1 medium on Matrigel. IMR90 human fetal lung fibroblasts (passage 15–20), human dermal fibroblasts (primary isolated, passage 2) were cultured in DMEM with 1% l-glutamine, 1% non-essential amino acids, 10% FBS and 1% penicillin/streptomycin. Immunostaining and flow cytometer measurements were performed using antibodies listed in Supplementary Table 1.

Neural cell and cardiomyocyte derivation. Neural rosettes and neural progenitor cells were manually isolated from neural rosette cultures and propagated as an adherent monolayer on Matrigel in neural proliferation medium. After several manual passages with a cell scraper, confluent cultures of hiPSC-derived neural progenitor cells on Matrigel (1:200) were differentiated for 2 weeks into mature, β-III tubulin (TUJ1)/MAP2-positive neuronal cells through removal of FGF2 from the neural proliferation medium and change of medium every 2–3 d. hiPSC (IMR90)-derived cardiomyocytes were a kind gift from C. Xu (Emory University) and were derived as previously reported. Cells were cultured in RPMI/B27 medium and exhibited spontaneously contractile activity (Supplementary Video 3).

Design and fabrication of PDMS micropatterned arrays. Poly(dimethylsiloxane) (PDMS) micropattern arrays having islands with diameters of 10 μm, 20 μm, 56 μm and 170 μm were fabricated from silicon array masters. Microcontact printing on glass coverslips coated with Ti (100 Å) followed by Au (100 Å) was achieved using hexadecanethiol/(HO(CH₂CH₂O)₃-(CH₂)₃-SH) chemistry. Coverslips were incubated with ECM proteins (fibronectin or laminin, 50 μg mL⁻¹ in PBS). After a blocking step with 1% heat-denatured bovine serum albumin (Sigma) for 30 min and protein elution for 2 h in PBS, single-cell suspensions of IMR90 cells or hiPSCs were seeded in mTeSR1 medium with ROCK inhibitors Y27362 (10 μM, Calbiochem) or thiazovivin (2 μM, Stemgent).Briefly, hiPSCs were treated with 0.05% trypsin for 1 min and scraped as intact colonies. Cells were then prepared as single cells in mTeSR1 with Y27362 ROCK inhibitor and seeded as 100,000 cells mL⁻¹ on the micropatterned islands overnight. For unpatterned surfaces, glass coverslips were incubated with ECM proteins as above and single-cell suspensions were seeded as 60,000 cells mL⁻¹ and cultured overnight.

Cell adhesion strength measurements. Cell adhesion strength was measured using a spinning disk system. Coverslips with adherent cells cultured overnight were spun in PBS with 2 mM dextrose for 5 min at a constant speed in a custom-built device in compliance with American Society for Testing and Materials (ASTM F2664-11). The applied shear stress (τ) is given by the formula τ = 0.8μr(ρω²)¹/², where r is the radial position, ρ and μ are the fluid density and viscosity, respectively, and ω is the spinning speed. After spinning, cells were fixed in 3.7% formaldehyde, permeabilized in 0.1% Triton X-100, stained with DAPI (Life Technologies) and counted at specific radial positions using a ×10 objective lens in a Nikon TE300 microscope equipped with a Luddl motorized stage, Spot-RT camera and Image Pro analysis system. Sixty-one fields were analyzed, and cell cluster counts were normalized to the number of cell cluster counts at the center of the disk, where the applied force is 0. The fraction of adherent cells (f) was then fit to a sigmoid curve f = 1/(1 + exp(b(t - τₕ₅₀))), where τₕ₅₀ is the shear stress for 50% detachment and b is the inflection slope. τₕ₅₀ represents the mean adhesion strength for a population of cells. The adhesion-strength response was analyzed on micropatterned islands coated with fibronectin or laminin (50 μg mL⁻¹) or Matrigel (1:80).

Focal adhesion assembly. Immunofluorescence staining of focal adhesions was performed as previously described. Briefly, cells were prewashed with ice-cold PBS with calcium and magnesium, incubated in ice-cold cytoskeleton stabilization solution and fixed with 3.7% formaldehyde for 10 min. Immunofluorescence staining was performed using antibodies listed in Supplementary Table 1.
buffer (50 mM NaCl, 150 mM sucrose, 3 mM MgCl₂, 1 µg mL⁻¹ aprotinin, 1 µg mL⁻¹ leupepin, 1 µg mL⁻¹ peptatin and 1 mM phenylmethylsulfonyl fluoride) for 1 min and incubated twice (1 min each) in cytoskeleton buffer supplemented with 0.5% Triton X-100. Detergent-extracted cells were fixed in 4% paraformaldehyde in PBS, washed with PBS, incubated with a primary antibody against vinculin (Upstate) or talin (Sigma) and detected with Alexa Fluor 488–conjugated antibodies (Life Technologies).

Fabrication of microfluidic devices. PDMS (Sylgard 184, Dow Corning) microfluidic devices were fabricated as reported earlier using a negative photore sist (SU-8 2050, 50-µm thickness, MicroChem) and UV photolithography. Patterned negative molds were then exposed to vapor-phase tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (United Chemical Technologies) in a vacuum desiccator to prevent adhesion of PDMS. A 5-mm-thick layer of degassed PDMS mixture (10:1) was cast onto the mold and cured at 70 °C for 2 h. Cast PDMS devices were peeled off and then punctured for inlet-outlet holes and bonded to glass coverslips by exposure to oxygen plasma for 20 s.

μSHEAR-based isolation. Prior to coating with ECM proteins, the microfluidic channels and tubes were cleaned with 70% ethanol and rinsed thoroughly with PBS. ECM proteins at 50 µg mL⁻¹ (fibronectin or laminin) or 1:80 Matrigel were flowed through sterile devices and incubated for 1 h at room temperature. Small colonies of pluripotent stem cells and single-cell suspensions of fibroblasts were premixed and pipetted into the inlet reservoir using a 200-µL pipette tip and were cultured in the device for 24 h at 37 °C and 5% CO₂ before the detachment experiments. The device inlet was connected to a syringe pump using polyethylene tubing (BB31695-PE/4, Scientific Commodities), and outlet tubes emptied into collecting tubes. PBS was flowed at predetermined flow rates through the device to match up the desirable fluid shear stress, and cell detachment was monitored through a Nikon TE microscope. For this microfluidic flow configuration, the applied wall shear stress (τ) is defined by the formula τ = 12(µQ/wh²), where w and h are the width and height of the channel, respectively, µ is the fluid viscosity and Q is the fluid flow rate. Cells and colonies were plated on Matrigel-coated tissue culture plates in 10 µM ROCK inhibitor Y27362 (or 2 µM thiazovivin) containing mTeSR1 medium. For flow cytometry studies to determine purification efficiency, collected colonies or cells were immediately resuspended in a suspension of StainAlive DyLight 488 mouse anti-human TRA-1-60 antibody (Stemgent) and CMPTX CellTracker Red dye, stained for 45 min, washed and analyzed using an Accuri flow cytometer (BD Biosciences).

Pluripotent stem cell characterization. Karyotype analysis was performed on 20 metaphase spreads for each sample by CellLine Genetics. To determine population doubling time and survival, we dissociated detached colonies from μSHEAR into single cells and plated them in Matrigel-coated 12-well plates. At predetermined times, wells were washed and cells were counted. Embryoid bodies (EBs) from detached and expanded hiPSCs were formed using an ultrahigh-throughput forced aggregation method and, after 24 h, cell aggregates were transferred to a suspension culture on a rotary orbital shaker (65 r.p.m.) for 14 d. After differentiation, we plated EBs in cell chambers (BD Falcon), and after 21 d in chambers, differentiated cells were fixed with 4% paraformaldehyde, permeabilized with 0.05% Triton X-100 and stained with antibodies against α-fetoprotein, α-smooth muscle actin and PAX6.

Gene expression analysis. RNA was isolated from iPSCs using QIAshredder and RNeasy Mini kits (Qiagen) according to the manufacturer’s protocols. We performed first-strand cDNA synthesis with the RT2 First Strand Kit (SA Biosciences) followed by real-time PCR, using the Human Embryonic Stem Cells PCR array (SA Biosciences) according to the manufacturer’s recommended protocols and using a BioRad MyCycler and BioRad MyIQ real-time thermal cycler, respectively. Individual Ct values were first internally normalized to GAPDH and subsequently analyzed with Genesis software (Graz University of Technology), including log transformation and hierarchical clustering. Heat maps were generated for the expression of 84 embryonic stem cell–related genes for transcription factors, pluripotency and self-renewal, cytokines and growth factors; and embryonic stem cell differentiation/lineage marker genes extracted from gene expression microarrays. A log plot of the relative expression level of each gene (2−ΔCt) between manual (x axis) and μSHEAR (y axis) was generated for analysis.

Bisulfite genomic sequencing. Bisulfite treatment of gDNA was carried out using a Cells-to-CpG Bisulfite Conversion kit (Life Technologies) according to the manufacturer’s protocol and plotted as melting curves representing methylation status. Converted gDNA was amplified by PCR using primers within the OCT4, SOX2 and NANO promoter genes. Primer sequences are as follows: OCT4 forward: CCTCCCTCTAAAAAAC; OCT4 reverse: GGGTTTGATGGTGTGGTATTAT; NANO forward: AA TTACAAAAATACCCCC; NANO reverse: TAGTTGGAATTCACAAAT; SOX2 forward: CATACACACATAAAAA; SOX2 reverse: GTTTTTTGGTGATTTTGG.

Teratoma formation. The μSHEAR-isolated cells were expanded on Matrigel and then collected as pellets resuspended in DMEM-F12 at 7 million cells per 50 µL. Cells were injected intramuscularly in the hind limb of SCID mice (Harlan). Seven weeks after injection, tumors were dissected, weighted and fixed with formalin. Paraffin–embedded tissue was sectioned and stained with hematoxylin and eosin (H/E) and imaged using a Nikon 80i microscope. All experimental and surgical procedures involving animals were approved by the Georgia Institute of Technology’s Institutional Animal Care and Use Committee.

Statistics. Paired two-tailed Student’s t-tests were performed to determine the significance of differences between two groups in adhesion blocking, adhesion strength and μSHEAR assays. For integrin profiling, one-way analysis of variance (ANOVA) was performed with Bonferroni correction using OriginPro 8.5.1. In all tests, P < 0.05 was regarded as statistically significant. All experiments were repeated in triplicate unless otherwise stated, and bar graph data represent average ± s.d.

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