Direct observation of mammalian cell growth and size regulation
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The doubling time of bulk culture as measured by the Coulter Counter was $11.4 \pm 0.7$ (s.d.) hours which is in agreement with the interdivision time of $11.3 \pm 1.5$ (s.d.) hours for single cells grown in the SMR chip.
Supplementary Figure 2 | Hydrodynamic focusing constrains the flow path of a cell that transits the SMR

The cartoon shows the pressure configurations in the four ports: high pressure (dark grey) and low pressure (light grey). Solid black lines indicate a fixed pressure and dashed black lines indicate an alternating pressure. If a pressure gradient exists along the bypass channel, then particles that enter the SMR will travel through it with a highly variable flow path. This is evident when the resonant frequency shift from a 12 μm polystyrene bead is repetitively measured (black points on plot). By balancing the pressure along the bypass, flow enters the SMR from the top and bottom sides and the particle’s path is focused. The dashed red line in the cartoon indicates focusing through the outer flow path (red points on plot) and the dashed blue line indicates focusing through the inner flow path (blue points on plot).
Supplementary Figure 3 | Growth rate acceleration ratio at the G1-S transition

Histogram shows the distribution of the slope ratio, which is defined by the growth rate acceleration just after the G1/S transition divided by acceleration just before the G1-S transition. The transition point was determined by a bilinear curve fit (Online Methods). There were 61 cells (out of 122) that exhibited slope ratios below 0.3 and 25 cells with ratios between 0.3 to 0.7. Inset shows two representative trajectories: 1 slope ratio of 0.22, 2 slope ratio of 0.34.
The black lines show the mean growth trajectory from all cells in a particular lineage and the blue shaded region is defined by three times the coefficient of variance. Inset: The mean (black line) and standard deviation (blue area) of the growth rate-per-mass versus mass for the lineage.
A negative correlation becomes more apparent when cells are grown under limited isoleucine conditions. Early G1 growth rate is determined by averaging the mass accumulation rate between the first and third hour following cell division. Normal: $n = 49$, Pearson’s correlation coefficient $= -0.75$; Limited isoleucine: $n = 16$, Pearson’s correlation coefficient $= -0.83$. 
Supplementary Figure 6 | Interdivision and G1 time versus newborn cell size

(a) L1210 mouse lymphoblast cells. Blue and red circles: Time at G1/S transition versus mass at birth (blue \( n = 77 \), Pearson’s correlation coefficient = 0.03, red \( n = 18 \), Pearson’s correlation coefficient = 0.10). Grey circles: time at division versus mass at birth (\( n = 122 \), Pearson’s correlation coefficient = 0.08). (b) FL5.12 pro-B-cell lymphoid cells. Black circles: time at G1/S transition versus mass at birth (\( n = 28 \), Pearson’s correlation coefficient = –0.42), Grey circles: time at division versus mass at birth (\( n = 28 \), Pearson’s correlation coefficient = –0.36).
Supplementary Figure 7 | Lineage growth trajectories for L1210 and FL5.12 cells
Representative growth curves of lineages grown in different environments. The black line indicates cells grown in normal media and the blue line under limiting amount of Isoleucine. The columns show the mean size of cells at birth, G1–S transition and division. n = 77 for normal; n = 17 for limited isoleucine.
G1 (red) or post G1 (blue) cells are measured from a steady-state cell culture. Black line indicates entire population. (a) normal media (12 h doubling time), (b) limited isoleucine media (19 h doubling time).
Supplementary Figure 10 | Lineage trajectories for cells grown in normal and media with limited isoleucine
(a) One upstream and two downstream vials are pressurized by independent pressure regulators. The system is primed with media (blue) before a cell is loaded. During cell loading, only the downstream vial on the right is pressurized (6 psi) and the sample plug (red) flows into the chip. The flow rate is qualitatively shown with the thick (high) and thin (low) arrows. (b) As the sample plug moves toward the SMR, an equal pressure (3 psi) is applied to both the upstream and downstream vials. By balancing the pressure applied across the bypass, fluid from both the upstream and downstream directions enter the SMR. As a result, the flow path of the cell is confined to either the inner or outer region of the channel, which minimizes the position dependent error (see Supplemental Figure 2). (c) After the sample has been completely loaded, all pressure sources are turned off and the sample vial is exchanged to media. The downstream vial is then pressurized again to keep the cell in transit. (d) As soon as a cell of a desirable size transits through the SMR, the flow direction is reversed and the flow rate is reduced by elevating the downstream pressure to 2.9 psi. The sample plug on the right bypass channel is rinsed away by flow from the upstream direction. The liquid level in all three vials is matched in order to remove any leakage flow induced by variations in hydrostatic pressure.
Supplementary Figure 12 | SMR optical system for single cell fluorescence measurement
(a) Mean and standard deviation of the fluorescent signals from three different populations of MESF standard beads. Linear fit (red, \( r^2 = 0.998 \)) shows the sensitivity of \( 5.8 \times 10^{-7} \)/MESF. The corresponding baseline noise for three different signals are shown at the bottom of the plot (one sigma, acquired at 10Hz bandwidth). The signal-to-noise ratio is 70, 130, and 210, for the \( n = 61, 108, \) and 102 measurements, respectively. (b) The fluorescent signal of the same bead is repeatedly measured every 3 seconds for \(~1\)hr. The signal exponentially decays due to photo-bleaching. The RMSE is 1%.
The pH in the bypass reservoir was measured over time for four cell transit conditions and two SMR chip designs (see Table). Transit conditions were determined by how frequently the cell transited through the SMR, which together with the bypass-to-buried surface area ratio, determined the media replenishment rate in the chip. pH in the chip slowly increased when the replenishment rate was too low due to the local leakage of CO₂ that occurs in the tubing-to-chip gasket interface. For the design with the low bypass-to-buried-surface-area ratio, it was necessary to transit the cell every minute in order to maintain constant pH (black filled circles). However, for FL5.12 cells, this level of shear stress was detrimental. By using a SMR chip with a larger bypass-to-buried-surface-area ratio, the pH was stably maintained over extended periods of time with a low transit frequency (black open circles).

**Table**: pH stability in the bypass reservoir

<table>
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<th>Time (h)</th>
<th>pH</th>
<th>Freq. of transit (hr⁻¹)</th>
<th>Bypass/Buried Surface area</th>
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</table>

**Diagram**

Supplementary Figure 14 | pH stability in the bypass reservoir