Supporting Information

Sorting single satellite cells from individual myofibers reveals heterogeneity in cell-surface markers and myogenic capacity

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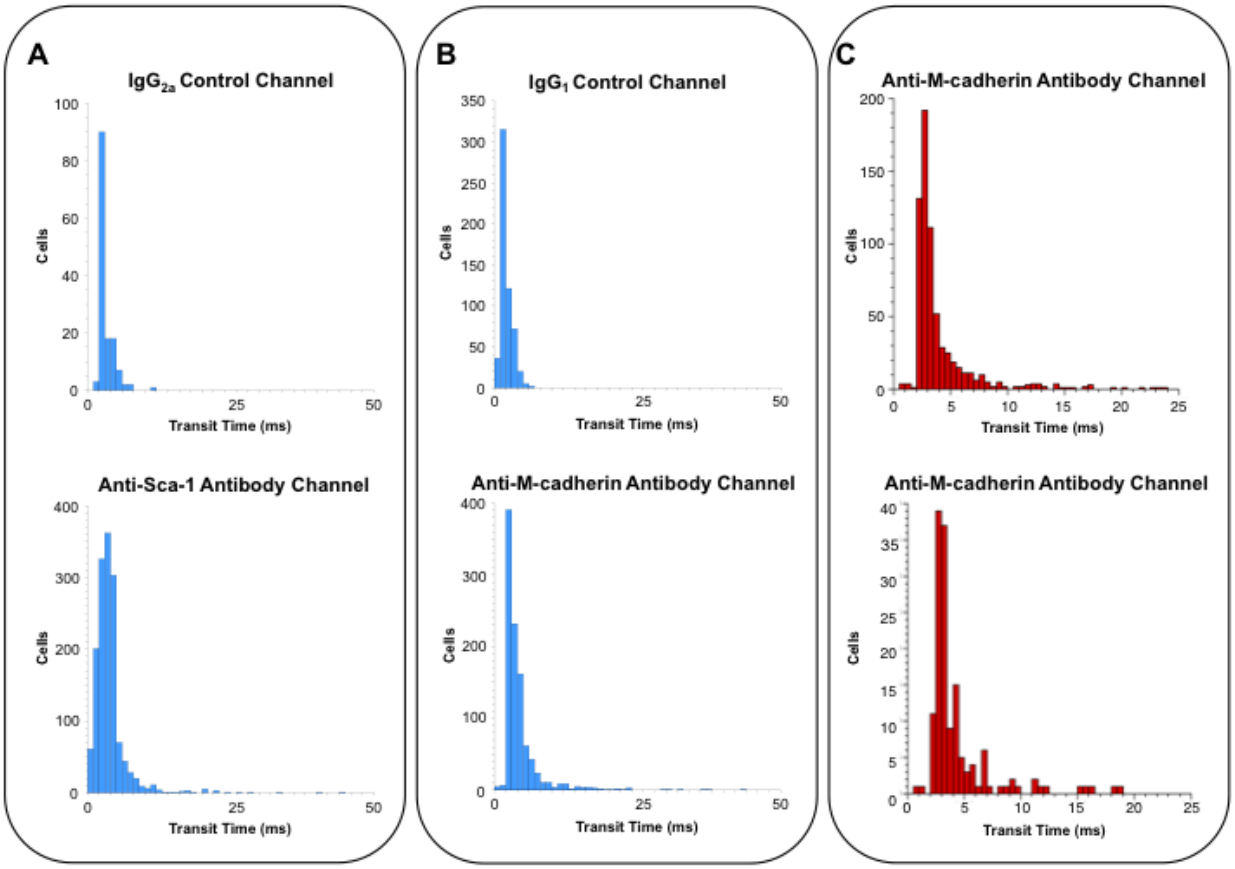


Figure S1: Screening primary-culture mouse myoblasts with functionalized microchannels. (A) Transit-time (τ) distribution of myoblasts screened with IgG_{2a} isotype-control (number of cells, n = 141, τ_{avg} = 3.16 ± 0.13 ms) and anti-Sca-1 (n = 1469, τ_{avg} = 3.89 ± 0.35 ms) antibody-coated microchannels, respectively. (B) τ-distribution of myoblasts screened with IgG_1 isotype-control (n = 218, τ_{avg} = 1.44 ± 0.55 ms) and anti-M-cadherin (n = 1004, τ_{avg} = 4.45 ± 3.83 ms) antibody-coated microchannels, respectively. Using a False Discovery Rate (FDR) analysis, 2.7% of cells screened were found to be Sca^+ and 93.0% were found to be M-$cadherin^+$ (see SI *Extended Experimental Procedures* for analysis details). (C) τ-distributions of the same population of myoblasts screened with two different microchannels functionalized with anti-M-cadherin antibody. The distributions are nearly identical (top distribution: n=672, τ_{avg} = 4.16 ± 3.00 ms; bottom distribution: n=146, τ_{avg} = 4.38 ± 3.00 ms), thereby demonstrating that the screening method is accurate and reproducible.

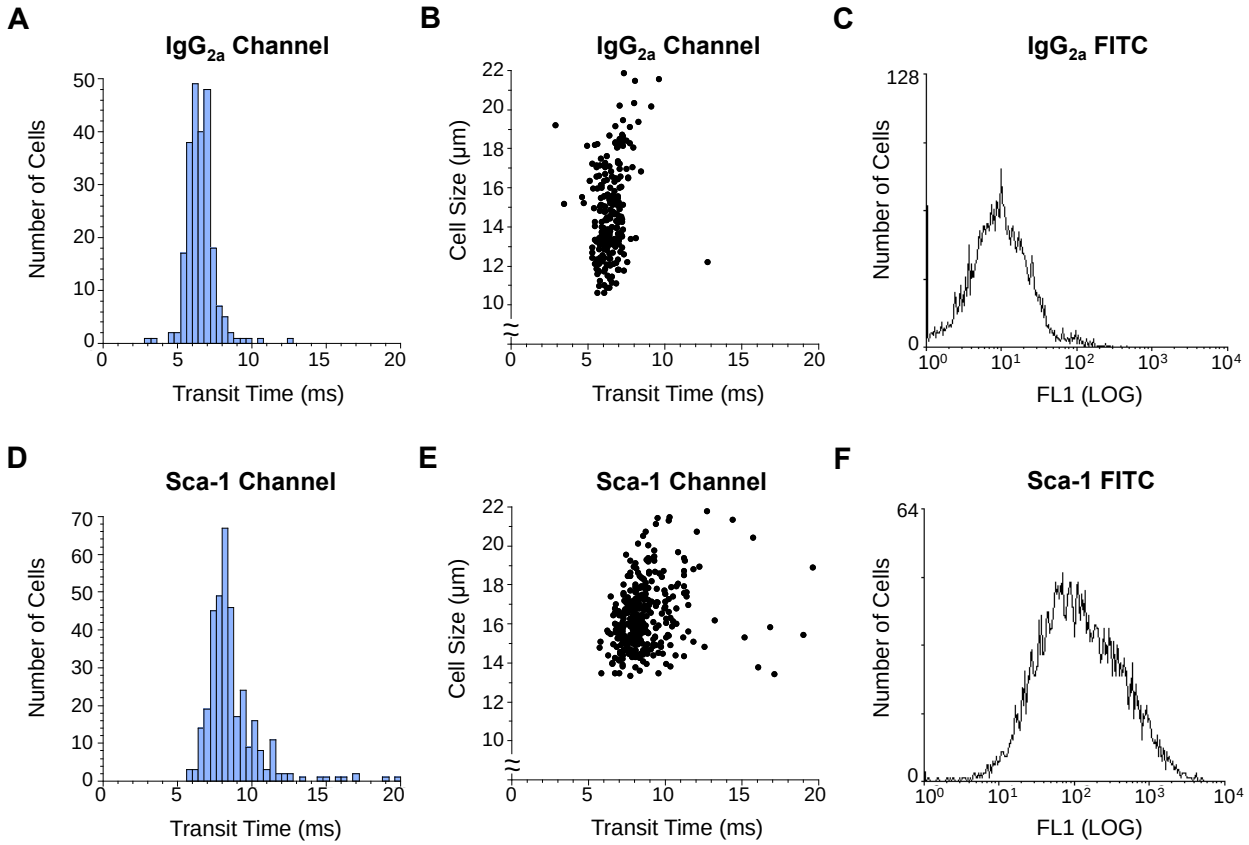
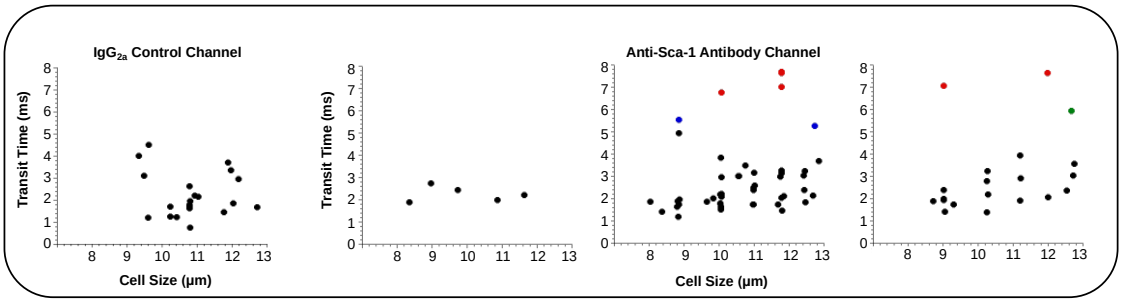
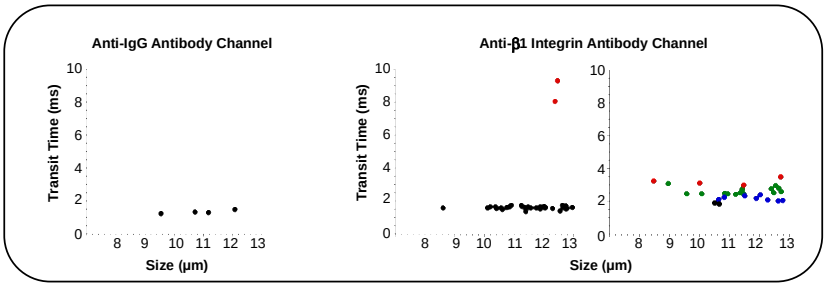
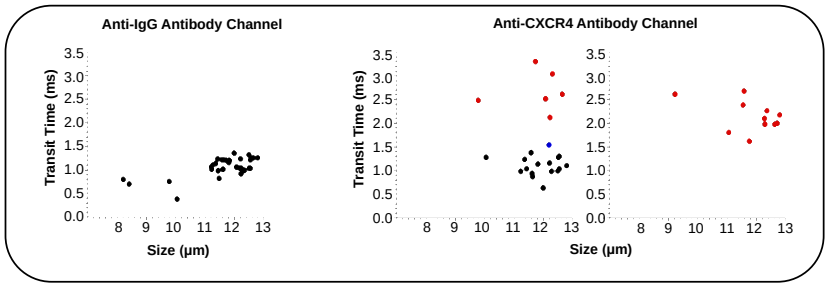
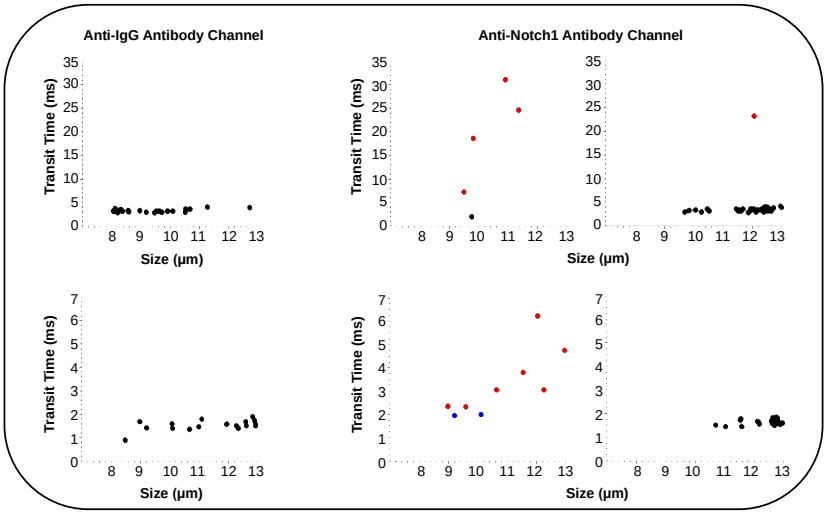


Figure S2: Screening Sca-1^+ cells with functionalized microchannels. **(**A**)** and **(**D**)** τ-distribution of a bulk population of satellite cells derived from uninjured (tibialis anterior and gastroc) muscle**.** Cells transited through microchannels functionalized with either an IgG_{2a} antibody **(**A**)** or anti-Sca-1 antibody **(**D**)**. Using an FDR calculation, 67.6% of the cells were found to be Sca-1^+. **(**B**)** and **(**E**)** Scatter plot of cell size vs. transit time for cells transiting an IgG_{2a} and anti-Sca-1 antibody microchannel, respectively. In the scatter plots, $R^2 = 0.12$ and 0.06 for **(**B**)** and **(**E**)**, respectively, indicating no correlation between cell size and transit time. **(**C**)** and **(**F**)** FACS histograms of the same population of cells screened with our microchannel described in **(**A**)** and **(**B**)**. **(**C**)** corresponds to cells immunostained with control FITC-labeled IgG_{2a} while **(**F**)** corresponds to cells immunostained with FITC-labeled anti-Sca-1 antibody. FACS analysis determined that 66.1% of cells in the population are Sca-1^+.


IgG2a Control Channel
Anti-Sca-1 Antibody Channel
Transit Time (ms)
Cell Size (µm)


Anti-IgG Antibody Channel
Anti-β1 Integrin Antibody Channel
Transit Time (ms)
Size (µm)


Anti-IgG Antibody Channel
Anti-CXCR4 Antibody Channel
Transit Time (ms)
Size (µm)


Anti-IgG Antibody Channel
Anti-Notch1 Antibody Channel
Transit Time (ms)
Size (µm)

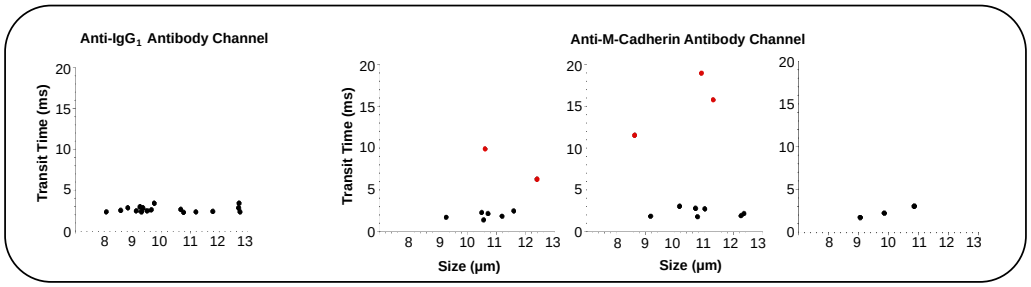

Anti-IgG1 Antibody Channel
Anti-M-Cadherin Antibody Channel
Transit Time (ms)
Size (µm)

Figure S3: Transit time of freshly isolated satellite cells for different experiments. Sca-1^+, β1-integrin$^+$, CXCR4^+, Notch-1^+, and M-cadherin$^+$ cells were identified by statistically assessing whether a particular cell had an outlying slow transit time as compared to those cells passing through the isotype control microchannel (p-values were calculated). Under different p-value cutoffs, cells can be considered as having a high expression (red), medium expression (green), low expression (blue), or no expression (black) (see SI *Extended Experimental Procedures* for analyses details).

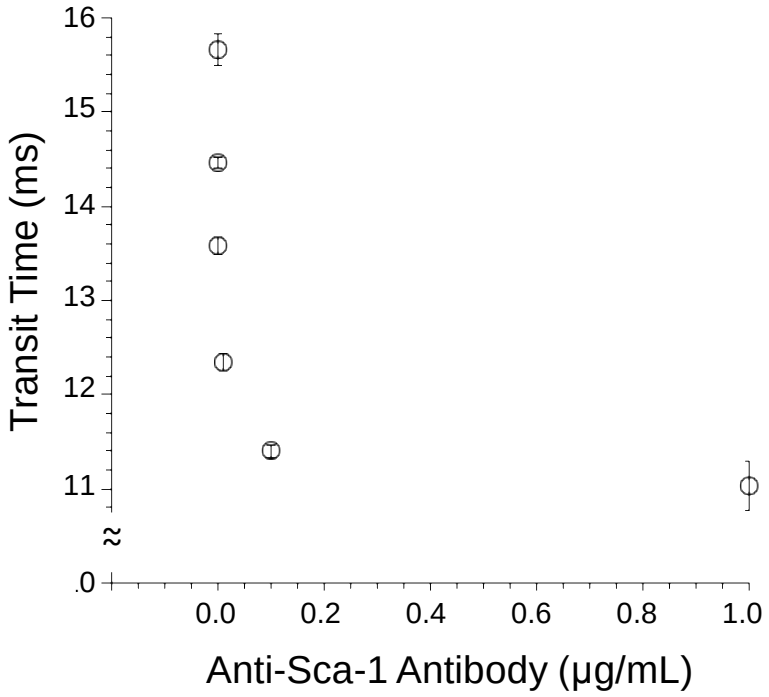


Figure S4: Transit time of Sca-1$^+$ cells incubated with an increasing concentration of anti-Sca-1 antibody. As more antibodies bind to the Sca-1 receptors on the cells, fewer receptors are available to bind transiently to the anti-Sca-1 antibodies (0.20 mg/mL) functionalized in the channel. Correspondingly, the transit time decreases logarithmically. The error bars correspond to the standard error.

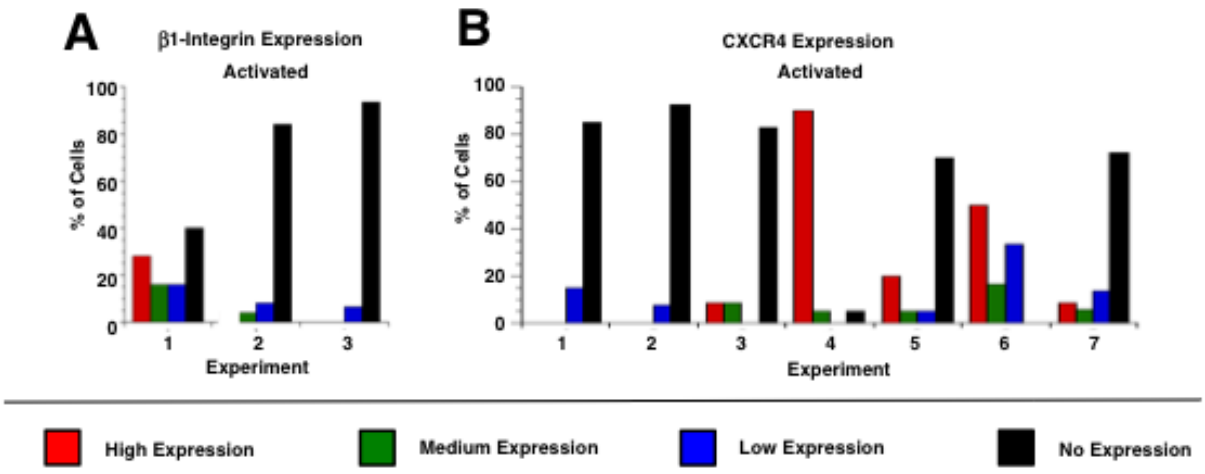


Figure S5: Screening analysis of cultured activated satellite cells derived from single muscle fibers. (A) β1-integrin marker screening. Each experiment consisted of cells collected from 3 single muscle fibers and screened with a different β1-integrin antibody microchannel, each 800 μm-long. Based on Dixon's Q Test (see Supporting Information Materials and Methods for analyses details), cells were determined to have high (red), medium (green), low (blue), or no (black) β1-integrin expression. Experiments 1, 2, and 3 consisted of n=25, 25, and 15 cells, respectively. Wide heterogeneity in the expression of the β1-integrin marker is evident from fiber to fiber. (B) CXCR4 marker screening. 800 μm long microchannels were used for Experiments 1-3 (n=20, 27, and 35 cells, respectively); 2000 μm long microchannels were used for Experiments 4-7 (n=20, 20, 6, and 36 cells, respectively). All markers screened show a wide range of heterogeneity.

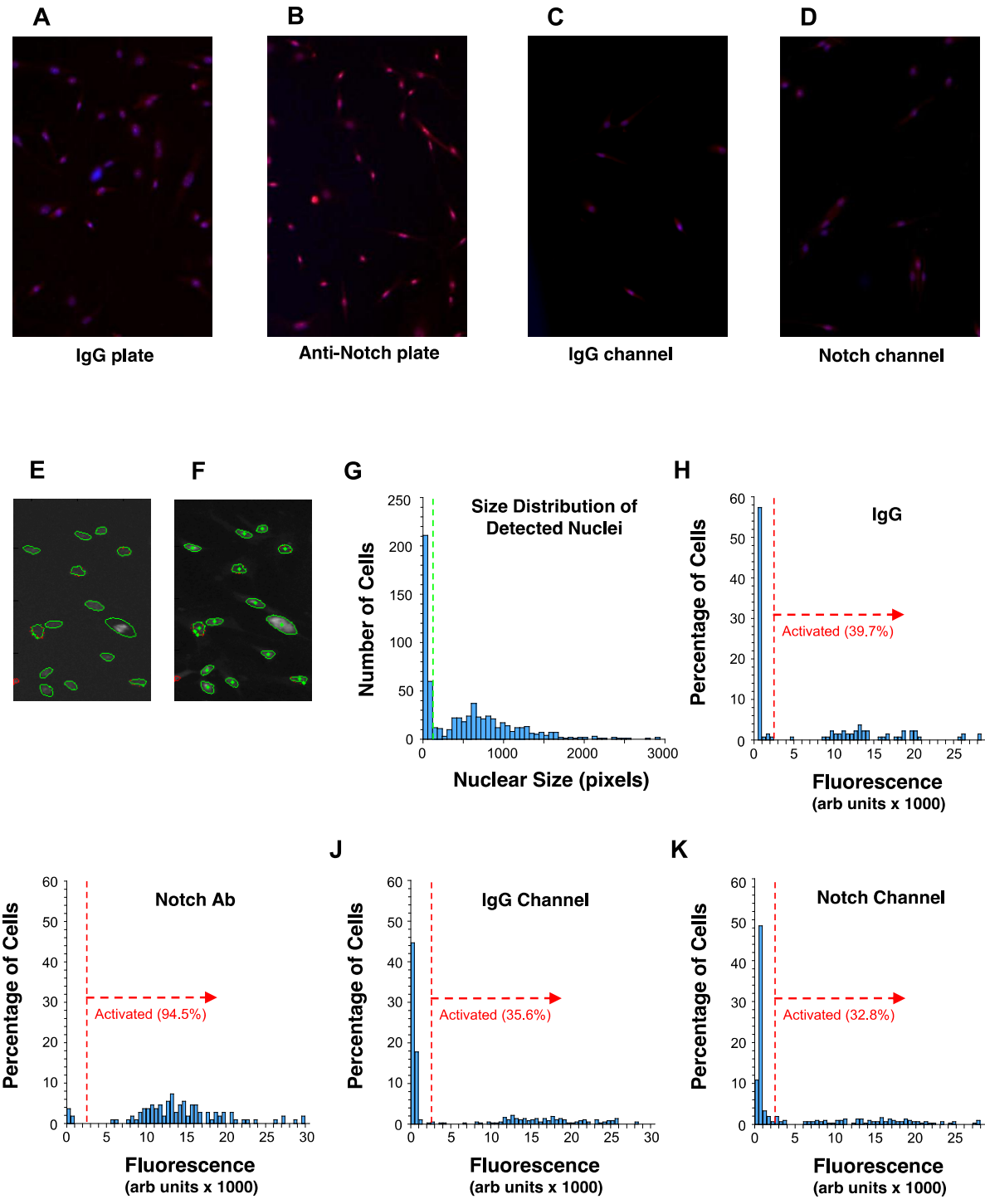

A
B
C
D
IgG plate
Anti-Notch plate
IgG channel
Notch channel
E
F
G
Size Distribution of Detected Nuclei
Number of Cells
Nuclear Size (pixels)
H
IgG
Activated (39.7%)
Percentage of Cells
Fluorescence
(arb units x 1000)
I
Notch Ab
Activated (94.5%)
J
IgG Channel
Activated (35.6%)
K
Notch Channel
Activated (32.8%)

Figure S6: Analysis of cells for Notch activation. **(**A-D**)** Representative images of the indicated culture conditions that were analyzed to determine the percentage of cells with high levels of nuclear notch. The red corresponds to Notch, and the blue to the nuclear Hoechst stain. **(**E**)** An example of a grey-scale image of the Hoechst nuclear stain with the final boundaries determined by our MATLAB program traced in green. Traces in red are identified points that were discarded due to either small size or proximity to the image boundary. Several of the boundaries are larger than the area of the nuclear stain for that cell. This is due to the addition of the detected area of Notch fluorescence identified in (F), which shows the grayscale image of Notch fluorescence corresponding to the nuclear stain in (E). (G) The histogram of detected nucleus size for the growth media control. The vertical dashed green line indicates the cutoff for boundaries that are too small and most likely correspond to fluorescent debris or random noise in the image. Boundaries encompassing less than 120 pixels (i.e. to the left of the green line) were discarded. **(**H-K**)** Histograms of fluorescence intensity due to nuclear notch. Cells with a total fluorescence intensity of >2500 were considered to have high levels of nuclear Notch, and thus activated. The vertical dashed red lines indicate the cutoff for cells that are considered to be activated.

Supporting Information Materials/Methods

Harvesting of satellite cells from single muscle fibers: Un-injured EDL muscle was dissected from the hind leg of 3-month old C57/black-6 mouse and incubated at 37^{o}C in digestion medium (250 U/mL Collagenase type II (Sigma) in DMEM, buffered with 30 mM HEPES, pH 7) for 1 hour with gentle agitation [1]. Digested muscle was gently triturated through a flame-polished Pasteur pipet, fibers were settled briefly and pipetted to a tissue-culture dish in DMEM/F12 with 2 % horse serum. Single muscle fibers were hand picked under a microscope and satellite cells were liberated from three single fibers by digestion for one hour, with 1 U/mL Dispase (Gibco#17105-014) and 40 U/mL Collagenase type II in medium). Freshly isolated satellite cells were directly analyzed through the microfluidic device.

Culture of activated satellite cells from single muscle fibers: Un-injured EDL muscle was dissected from the hind leg of 3-month old C57/black-6 mouse and incubated at 37^{o}C in digestion medium (600 U/mL Collagenase type I (Sigma) in DMEM, buffered with 30 mM HEPES, pH 7) for 2 hours with gentle agitation [1].. Digested muscle was gently triturated through a flame-polished Pasteur pipet, fibers were settled briefly and pipetted to a tissue culture dish in plating medium (DMEM/F12 with 2 % horse serum) for culture and activation overnight. Single fibers were hand picked under microscope and cultured in myoblast growth medium [DMEM/F12 with 10 % Bovine Growth Serum (Hyclone) and 5 ng/mL FGF-2], to further activate satellite cells for two days. Satellite cells were liberated from three single fibers by digestion for one hour, with 1 U/mL Dispase (Gibco) and 40 U/mL Collagenase type II in medium before analyzed with microfluidic devices.

Isolation and sorting of Sca-1-GFP myoblasts, and immunofluorescence staining: Fresh Sca-1-GFP muscle from a transgenic mouse [2] was digested and bulk myofibers with satellite cells were isolated as published [3]. Bulk fibers were cultured in myoblast growth medium (DMEM/F12 with 10 % Bovine Growth Serum (Hyclone) and 5 ng/mL FGF-2) for three days; myogenic cells were prepared from the culture as published [3], and cultured for an additional day in growth medium on dishes coated with diluted Matrigel in PBS (3 μg/mL/cm^2). Myogenic cells were washed and lifted in PBS and live sorted by FACS on GFP for three populations: low/no GFP (21 %), intermediate GFP (34 %) and high GFP (17 %). The three populations were plated in Matrigel-coated chamber slides and cultured in growth medium overnight, then cultured in differentiation medium (2 % horse serum in DMEM) for four days. Cells were then imaged for GFP fluorescence, fixed in 4 % paraformaldehyde (5 minutes), and immunostained for embryonic myosin heavy chain (eMyHC) and GFP, as published.

Preparation of microchannel devices: Negative-relief masters of the final microfluidic channel devices were fabricated on polished silicon wafers using standard soft lithography [4]. The microchannel's cross-sectional area, 25 μm x 25 μm, was chosen to be comparable to the size of the cells. The microchannel length of either 800 or 2000 μm ensured sufficient signal-to-noise ratio and transit-time resolution in our measurements [5]. PDMS (Sylgard 184) (10:1, pre-polymer : curing agent) was degassed and dispensed onto the master, and cured for 1 hour at 80°C. Prior to measurement, PDMS slabs with the embedded microchannel device were cut from each master. A 16 G syringe needle was used to core access holes into the PDMS slab.

Preparation of electrodes: The electrodes were lithographically patterned onto RCA-cleaned glass substrates using Microposit S1813 (Dow) resist. A 75/250 Å Ti/Pt thin film was deposited using an electron-gun evaporator. Before sealing to the PDMS slab, the glass substrates were functionalized.

Device functionalization and assembly: Glass substrates were cleaned in a 10:1:1 RCA solution heated to 80°C for 5 min, followed by a thorough rinse in DI water. To functionalize the glass substrate area between the electrodes with antibodies, a temporary PDMS microchannel, created with soft lithography, was aligned between the electrodes and hermetically (reversibly) sealed to the substrate. This microchannel served as a patterning guide and ensured that only the region between the electrodes was silanized. A solution of 1M sodium hydroxide (Fisher Scientific) was injected into the microchannel for 10 min. The solution was then removed from the microchannel, and the devices were heated to 150°C for 10 min. After cooling, a silane solution (2% N-3-triethoxysilypropyl-4-hydroxybutramide [Gelest] in 95% ethanol [Acros], 0.1% acetic acid [Fisher Scientific]) was injected into the microchannel and incubated for 4 hours. The glass substrate was rinsed with a stock solution of 95% ethanol and 0.1% acetic acid, DI water, and baked at 120°C for 2 hours. The prepared PDMS microchannel (see Preparation of microchannel devices) was subsequently aligned and bonded (via oxygen plasma and heat) to the substrate. The just-silanized region on the glass substrate was protected with a piece of PDMS during plasma treatment. After PDMS bonding, 10 µL of the cross-linker, Sulfo-EGS, (3 mg/mL, Pierce) was injected into the microchannel for 20 minutes. Protein G (1 mg/mL, Pierce) was subsequently injected into the microchannel and incubated for 2-5 hrs. After incubation, excess Protein G was washed away with PBS. Antibodies (Table S1) at a concentration of 0.2-0.25 mg/mL (depending on stock concentration from the supplier, see Table S1) were injected into the prepared microchannel and incubated overnight at 4°C. The microchannel was then flushed thoroughly with PBS, then media, and kept filled with media for cell screening.

Data acquisition and analysis: A four-point measurement of the current was performed using a constant applied AC voltage (typically 0.2–0.4 V), as previously published [5-7]. The current was passed through a preamplifier (DL Instrument 1211) that performed a low-pass filter at 0.3 ms in rise time. The resulting output was connected to a data acquisition board (National Instruments PCI-6035E) for data sampling at 50 kHz. Data was recorded and analyzed using custom-written software in LabView. The raw data (green trace in Figure 1D) underwent a three-point smoothing algorithm (red trace in Figure 1D), and the derivative of the resulting smoothed data was plotted to identify the boundaries of each pulse.

Determination of cell size: The relative height of a current pulse, $\delta I/I$, where δI is the absolute pulse magnitude and I is the baseline current, is dependent on the cell diameter, d, an effective diameter, D, of the microchannel, and the microchannel length, L, through the following (Saleh and Sohn, 2001, 2003a; Carbonaro et al., 2008; Chapman and Sohn, 2011),

$$\left|\frac{\delta I}{I}\right| = \frac{D}{L}\left[\frac{\arcsin\left(d/D\right)}{\sqrt{1-\left(d/D\right)^2}} - \frac{d}{D}\right] \qquad (1)$$

D was first determined experimentally by measuring the pulse magnitude of 15 um colloids (Bangs Laboratories) transiting the microchannel. The pulse magnitude was averaged over three

runs on three different devices made with the same master. The effective diameter of the microchannel was determined to be D= 24 μm using Eq. 1. We subsequently were able to determine d for each cell measured.

To convert the recorded dimensionless $\delta I/I$ value into cell size (μm), we make use of the Solver for Nonlinear Programming, which we install in OpenOffice by Oracle®. This NLP solver makes use of two evolutionary algorithms, Differential Evolution (DE) and Particle Swarm Evolution (PSE). For the purposes of this $\delta I/I$ value into cell size (μm) conversion, we specifically utilize the Particle Swarm Optimization algorithm, which represents solution vectors as coordinate "particles" in an n-dimensional "room". As each particle traverses through the "room", it evaluates and keeps track of the best solution so far. The information is shared with the entire particle swarm, allowing for further iterations to be influenced by these current best solutions. Eventually, the particle swarm converges and stagnates around the optimal solution.

Screening primary-culture mouse myoblasts for Sca-1 and M-cadherin expression: Primary-culture mouse myoblasts were isolated from freshly harvested mouse EDL muscle. Myoblasts were cultured in Matrigel- coated plates (2 μg/ml/cm^2) in Ham's F-10 media with 20% BGS, 1X penstrep, and 6 ng/ml FGF. Cells were dissociated from the matrigel in PBS with gentle agitation for 3-5 min to prevent digestion of surface receptors prior to screening. Figure S1 *A* and *B* shows the τ-distributions derived from cells transiting an isotype-control, mouse IgG_{2a} (n = 141, τ = 3.16 ± 0.13 ms, Figure S1 A) and rabbit IgG (n = 218, τ = 1.44 ± 0.55 ms, Figure S1 B) microchannel, respectively. Figure S1 *A* and *B* show the τ-distributions derived from cells transiting an anti-Sca-1 (n = 1469, τ = 3.89 ± 0.35 ms, Figure S1 A) and anti-M-cadherin (n = 1004, τ = 4.45 ± 3.83 ms, Figure S1 B) antibody-coated microchannel, respectively.

To identify Sca-1$^+$/M-cadherin$^+$ cells among the cells that passed through an anti-Sca-1/anti-M-cadherin antibody microchannel, we first estimated the empirical probability density function for all cells passing through an IgG_{2a} control microchannel. With the estimated probability density function serving as a null distribution, we next computed the p-value and corresponding False Discovery Rate (FDR) for each cell passing through an anti-Sca-1/anti-M-cadherin antibody microchannel. The FDR was computed as follows: Let $p(i)$ denote the ith largest p-value among all cells. We estimated the corresponding FDR (when $p(i)$ is used as a cutoff to determine Sca-1$^+$/M-cadherin$^+$ cells) by $m*p(i)/i$, where m denotes an (overly) estimated number of Sca-1$^-$ cells, e.g., m can be the total number of cells passing through the anti-Sca-1 antibody microchannel.

In a list of identified Sca-1$^+$/M-cadherin$^+$ cells, FDR controls the expected proportion of incorrect identifications of Sca-1$^+$/M-cadherin$^+$ cells (i.e., false positives) [8]. It is a less conservative procedure compared to the Bonferroni correction (see below), and especially preferred when a large number of tests are performed simultaneously.

Screening Sca-1$^+$ cells in a bulk population of cells: We compared the results of our screening method to those of FACS using satellite cells freshly harvested from mouse EDL muscle in bulk as published [9]. Figure S2 A and D show the τ-distributions derived from cells transiting through a control antibody, IgG_{2a}, microchannel (τ_{avg} = 6.44 ± 0.96 ms) and an anti-Sca-1 antibody microchannel (τ_{avg} = 8.62 ± 1.78 ms), respectively. The notable increase in τ_{avg} and the τ-distribution variance of cells transiting the anti-Sca-1 antibody microchannel is indicative of Sca-1$^+$ cells in the sample [6]. Transit-times are uncorrelated to cell size, as shown in Figure S2 B and

E. Statistically, cells whose transit times were significantly high (with a FDR [8] < 0.01, see below) as compared to the τ-distribution for the control antibody microchannel are Sca-1^+. Of the 349 cells measured in the anti-Sca-1 antibody microchannel, 67.6% were Sca-1^+. FACS analysis of the same population of cells determined that 66.1% of 10^5 cells screened were Sca-1^+ (Figure S2 C and F).

Identifying Sca-1^+ cells within a mixed population by controlling False Discovery Rate (FDR): To identify Sca-1^+ cells among the cells that passed through an anti-Sca-1 antibody microchannel, we performed a similar FDR analysis. The only difference from the previous analysis is that instead of using the empirical (i.e., non-parametric) probability density function, we used a parametric-way to estimate the probability density function (in a log-normal distribution family) for all cells passing through an IgG_{2a} control microchannel. We chose this parametric estimation because 1) the data fits the log-normal distribution very well and 2) there is less estimation of parameters involved so that the results are expected to be more robust. In our previous analysis for mouse myoblasts for Sca-1 and M-cadherin expression, the data does not seem to fit any parametric distribution we know and so a non-parametric estimation of the probability density function looks more appropriate.

A similar analysis was performed to identify Notch-1^+ cells within a mixed population (Figure 2C).

Relationship between surface-epitope density and transit time: To determine the relationship between the surface-epitope density and transit time through a microchannel functionalized with specific antibody, we performed a "blocking" experiment. A culture of Sca-1^+ cells (typically ~70% Sca-1^+ by FACS) was harvested from a plate by removing the growth media and incubating the cells in PBS for 3 minutes to dissociate the cells from the plate surface. The detached cells were spun down at 200 g for 5 minutes. The supernatant was removed, the cells were suspended in 3 mL of cell-staining buffer (Biolegend) and finally placed on ice. Purified Sca-1 antibodies (Biolegend) were mixed in concentrations of 1 μg/mL, 0.1 μg/mL, 0.01 μg/mL, 0.0005 μg/mL, and 0.0001 μg/mL in staining buffer. The Sca-1^+ cells were divided into 6 different tubes (500 μL each) for incubation with the antibody at the different concentrations (1 μg/mL, 0.1 μg/mL, 0.01 μg/mL, 0.0005 μg/mL, 0.0001 μg/mL and 0 μg/mL). The 6 tubes were spun down at 200 g and the supernatant removed. 100 μl of antibody was then added to the cells and incubated on ice for 30 minutes. After incubation, the cells were spun down and washed 3 times with PBS. Cells were then re-suspended in PBS with 2 % FBS and placed on ice for analysis using a prepared microfluidic device.

Devices were fabricated and functionalized with the Sca-1 antibody (Biolegend) at a concentration of 0.25 μg/mL, as previously described. A separate device was used for each incubation condition. Cells were injected into the device and passed through the microchannel at 6.9 kPa. A minimum of 100 cells was measured for each incubation concentration. The cell transit time was measured and Figure S4 shows the average time subsequently determined for each condition. As shown in the figure, there is a decreasing transit time vs. increasing incubating antibody concentration (to the point of saturation), thus demonstrating that our time-of-flight measurement not only detects the presence of a specific surface antigen, but also relates to the expression level of that particular antigen. Simply, an increased number of specific

surface receptors leads to an increased number of transient binding events to the functionalized antibodies, and consequently, longer transit times [6].

Significance (P-value) and False Discovery Rate (FDR) analysis for identifying Sca-1$^+$, M-cadherin$^+$, Syndecan-4$^+$, CXCR4$^+$, or Notch-1$^+$ cells: The p-values were calculated directly from null distributions of transit time. For each control sample (consisting of cells passing through the isotype control microchannel), a null distribution was estimated parametrically by fitting a Normal or Gamma distribution based on the empirical distribution shape of the control sample. Let $F_0(t)$ denote the estimated cumulative density function of null distribution for the control sample of, say Sca-1. Then the p-value for transit time t_x for a cell x passing through an anti-Sca-1 antibody microchannel can be calculated by 1- $F_0(t_x)$. We performed this p-value calculation on cells for all markers except β1-integrin. We categorized the cells into different expression groups as follows: cells whose transit times were significantly high (with raw p-values < 0.001 and Bonferroni corrected p-values < 0.05) were considered to have high levels of expression (Figures 2 and S3, red), while less stringent cutoffs on raw p-values of 0.01 and 0.05 were used to determine cells with medium (Figures 2 and S3, green) and low (Figures 2 and S3, blue) levels of expression of these markers, respectively. Note that the Bonferroni correction was applied here to address the problem of multiple comparisons, since we repeated the p-value calculation on all individual cells. This correction helped control the overall family-wise false positive discovery rate: if the probability of false positives is α for an individual test (i.e., the test at a significance level of α), the overall family-wise false positive discovery rate of n independent tests would be about $n\alpha$; the Bonferroni correction controls the family-wise probability of false positives by testing each of the individual tests at a significance level of α/n.

With the estimated null distributions, we further performed a False Discovery Rate (FDR) analysis to confirm our findings. Let p_0 be the p-value cutoff (e.g., $p_0 = 0.001$). Let m_1 be the number of cells with p-values less than p_0, and m_0 be the number of cells with p-values greater than a in the treatment sample. Then we estimate FDR at the p-value cutoff of p_0 as $m_0p_0/((1-a)m_1)$. We used a = 0.01 in our calculations. This generally provides an overestimation of FDRs. The estimated FDRs for cells with high levels of expression are all found to be < 0.01 (actually more than 80% of those cells have FDRs < 0.0001). The estimated FDRs for cells with medium or low levels of expression are mostly found to be < 0.02 and 0.05, respectively. These FDR results, to some extent, provide us a high confidence on the identified Sca-1$^+$, M-cadherin$^+$, Syndecan-4$^+$, CXCR4$^+$, or Notch-1$^+$ cells.

Dixon's Q Test for identifying β1-integrin$^+$ satellite cells: β1-integrin has a much smaller control sample (of size 4 only) compared to those of other markers. This makes it infeasible to estimate its null distribution of transit time and to perform a FDR analysis as above. Instead**,** we identified β1-integrin$^+$ associated satellite cells by running a Dixon's Q Test on each of the cells transiting an anti-β1-integrin antibody microchannel to determine whether the cell has a outlying slow transit time as compared to those transiting the IgG control microchannel. The details of this study were as follows. Let $x_1, x_2, \ldots, x_n$ be the transit time of the n cells passing through the IgG control microchannel, and let $y_{i1}, y_{i2}, \ldots, y_{ini}$ be the transit time of the n_i cells passing through an anti-β1-integrin antibody microchannel in experiment i. For every y_{ij} (the transit time of the jth cell in experiment i), we ran a Dixon's test on $x_1, x_2, \ldots, x_n, y_{ij}$ to detect if y_{ij} was an outlier compared to $x_1, x_2, \ldots, x_n$. For each experiment, the cells in red in Figure S3, with conservative Bonferroni corrected p-values < 0.05, are identified as high expressing β1-integrin$^+$ associated

satellite cells. Cells in green in Figure S3 corresponded to medium β1-integrin expression and had a raw p-value < 0.01. Further, cells in blue in Figure 3S corresponded to low β1-integrin expression and had a raw p-value < 0.05.

We note that the Dixon's Q test is a convenient and robust statistical test for identifying values that appear originated from a different distribution than the rest of the data, and it is particularly recommended for use in small samples [10]. The test statistic we used here took the ratio between the difference of the maximum value and its neighbor value and the difference of maximum and minimum values. We calculated the corresponding p-value by an existing R package.

Dixon's Q Test for identifying Sca-1$^+$ activated satellite cells: We similarly identified Sca-1$^+$ associated satellite cells by running a Dixon's Q Test on each of the cells transiting an anti-Sca-1 antibody microchannel to determine whether the cell has a outlying slow transit time as compared to those transiting the IgG_{2a} control microchannel. The details of this study were as follows. For each experiment, the cells with high expression (red, Figure 3A), with conservative Bonferroni corrected p-values < 0.05, are identified as Sca-1$^+$ associated satellite cells. The Bonferroni correction was applied here to address the problem of multiple comparisons, since we repeated the Dixon's Q test on all individual y_{ij}'s, $j = 1,\ldots,n_i$ in each experiment. This correction helped control the overall family-wise false positive discovery rate: if the probability of false positives is α for an individual test (i.e., the test at a significance level of α), the overall family-wise false positive discovery rate of n independent tests would be about $n\alpha$; the Bonferroni correction controls the family-wise probability of false positives by testing each of the individual tests at a significance level of α/n. Cells in green in Figure 3A corresponded to medium Sca-1 expression and had an *uncorrected* p-value = 0.01. Further, cells in blue in Figure 3A corresponded to low Sca-1 expression and had an uncorrected p-value = 0.05.

Similar analyses using the Dixon's Q Test and a Bonferroni correction were performed to determine high, medium, and low expressions of CXCR4$^+$, M-Cadherin$^+$, and β1-Integrin$^+$ activated satellite cells. The results are shown in Figures 2B (M-cadherin) and S5 (β1-Integrin$^+$ and CXCR4$^+$).

Notch receptor screening: Satellite cells were isolated from mouse muscle as described [3]. Briefly, muscle was dissected from the hind leg of 2-month old C57/black-6 mouse and incubated at 37ºC in digestion medium (200 U/mL Collagenase type II (Sigma) in DMEM, buffered with 10 mM HEPES, pH 7) for 2 hours with gentle agitation. After digestion the muscle was washed with PBS two times and triturated with a flame-polished broken pasteur pipette in buffered media (DMEM with 10% serum, buffered with 10 mM HEPES, pH7) in a shallow dish until the fibers were liberated. The dish containing the fibers was tilted to pool the liquid with fibers away from muscle chunks. The fibers were then transferred to a conical tube with additional buffered media and allowed to settle for 10 minutes, then washed two more times in buffered media before enzymatic digestion to liberate satellite cells (1 U/ml Dispase (Gibco), 40 U/ml Collagenase type II (Sigma) in DMEM, buffered with 10 mM HEPES, pH 7, for 1 hour with moderate agitation). The remaining debris was removed by gentle sedimentation (1 min, 200 x g), followed by filtration with a 40-50 μm mesh, and then the satellite cells were sedimented by centrifugation.

The isolated satellite cells were then screened with microchannel devices functionalized with either anti-Notch1 antibody (Invitrogen, 0.2 mg/mL) or the isotype control IgG_1 (Invitrogen, 0.2 mg/mL). 2000 μm long microchannels were used to ensure maximum affinity to the

microchannel. After screening, the cells were collected from the device and cultured in DMEM/F12 media with only 2 % horse serum so as not to further activate the cells. Cells from the original culture that were not screened with the microfluidic device were also plated overnight in 8-well plates coated with either anti-Notch1 antibody (Invitrogen, 0.2 mg/ml), which was a positive control for Notch activation, or the isotype control IgG_1 (Invitrogen, 0.2 mg/mL). Cells were fixed in ethanol and immunostained for activated Notch as described [11] (Figure S6 A-D). The number of cells with activated Notch was quantified by analyzing the fluorescence images using a custom-written program in Matlab: the Hoechst-stained image was converted to a binary format and the boundary of each nucleus was localized. The correlating Notch-stained image was then also converted to a binary format, and the boundary of nuclear Notch expression was localized. If the area of Notch expression was larger than the area of the nucleus, then all pixels within the boundary of Notch expression on the Notch-stained image are summed to generate a new boundary for that particular cell. If the area of the nucleus was larger than the area of Notch expression or no well-defined Notch-expression area could be identified for the corresponding nucleus, then all pixels that lie within the boundary of the nucleus in the Notch-stained image were summed. The boundaries were then transferred to the raw (i.e. not binary) image file and the fluorescence intensity inside each area determined to be a nucleus was summed (Figure S6 E and F). Then for each selected area, the average per pixel background for that image multiplied by the number of pixels was subtracted, leaving a final quantification of the amount of fluorescence per nuclei. In order to filter out any small particles or random noise, any detected area smaller than 120 pixels (Figure S6 G) was discarded. Cells that had a total fluorescence intensity of >2500 were considered to have high levels of nuclear Notch, and were subsequently considered to be activated (Figure S6 H-K).

Analysis of Sca-1 expression through immunofluorescence staining and microfluidic channel capture: Myofiber-associated satellite cells were isolated as published [3]. Cells were attached to matrigel-coated plates (2 μg/ml/cm^2) for 2 hours before immunostained for Sca-1 and Pax7. The cells were first live-stained for cell surface Sca-1 using Alexa 488 conjugated secondary antibodies (BD Biosciences), fixed in 4% paraformaldehyde, and washed and permeabilized before immunostaining for nuclear Pax7 (Developmental Studies Hybridoma Bank) as described [12]. .Fresh, isolated satellite cells were injected into a 100 μm x 2000 μm x 40,000 μm (H x W x L) microchannel functionalized with a saturating concentration of anti-Sca-1 antibody (Table 1) at a flow rate of 10 μL/min. Sca-1$^+$ cells became bound to the functionalized antibodies, and therefore captured. As a control, freshly isolated satellite cells were also injected into the same-sized microchannel functionalized with IgG_{2a} control antibody. As expected, no cells were captured with the control channel. Sca-1$^+$ cells captured in the anti-Sca-1 antibody channel were fixed in 4% paraformaldehyde, washed, and permeabilized before immunostaining for nuclear Myf5 (Santa Cruz Biotechnology).

Clonal analysis of single satellite cells: Single myofibers were isolated and analyzed with the devices. Immediately after screening for a pulse corresponding to a single satellite cell, the cell was collected in a separate well of 8-well cell culture dish. The cells were cultured in myoblast growth medium (DMEM/F12 with 10 % Bovine Growth Serum and 5 ng/mL FGF-2) for 14 days before immunostained for Pax7 and MyoD. Cell number, Pax7$^+$, MyoD$^+$, and Pax7$^+$/MyoD$^+$ cell percentage were quantified for each cell colony.

Table S1: Antibodies utilized in the described experiments.

Cell-Surface Marker (reactivity)	Antibody	Control Antibody	Source
Sca-1 (mouse)	Anti-Sca-1 (Ly-6A/E) 108102 (0.5 mg/mL)	Rat IgG_{2a}, κ (0.5 mg/mL)	Biolegend
CXCR4 (human/mouse/rat)	Anti-CD184 (CXCR4) ab2074 (1 mg/mL)	Rabbit polyclonal IgG (10 mg/mL)	Abcam
M-Cadherin (mouse)	Anti-M-Cadherin (12G4) ab78090 (1 mg/mL)	Mouse monoclonal IgG_1 κ (10 mg/ml)	Abcam
β1-Integrin (mouse/rat)	Anti-CD29 (β1-integrin) 102202 (0.5 mg/mL)	Armenian Hamster IgG (0.5 mg/mL)	Biolegend
Notch (mouse/rat)	Anti-Notch1 (8G10) sc-32756 (0.2 mg/mL)	Syrian hamster IgG (0.2 mg/mL)	Santa Cruz Biotechnology
Syndecan-4 (rabbit)	Anti-Syndecan-4 Ab24511 (0.5 mg/mL)	Rabbit polyclonal IgG (10 mg/ML)	Abcam

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