Supplementary Information

Fig. S1 Lab-on-a-disc device consists of a polyester film, polyacrylamide gel, and a microscope glass slide. The polyester lid encloses a cell suspension inside chambers during centrifugation. The microscope glass slide functions as a base support for the polyacrylamide gel and allows the imaging of cells and proteins during the device operation.

Fig. S2 A schematic workflow of the lab-on-a-disc handling and scWestern. 1: Centrifugal force and dams place single cells to microwells. Subsequently, cells settle in microwells by gravity. 2: After opening the polyester lid and cutting the device in half, a chemical lysis and electrophoretic buffer is poured on the device. Steps 3 – 5 entail scWestern. 3: An electric field (40 V/cm) is applied for protein separation. 4: UV is applied to activate benzophenone moieties, incorporated in the gel, for protein capture. 5: Fluorescent antibodies are introduced to probe for target proteins.
**Fig. S3** Rotational speed affects U251-GFP cells in the lab-on-a-disc. (A) Centrifugation at 3000 rpm for 5 min results in a mechanical lysis of U251-GFP cells. Cell debris are detected near microwells in brightfield and GFP channels. (B) U251-GFP cells are stained with propidium iodide and centrifuged in the lab-on-a-device at 2000 rpm for 2 min. Absence of red fluorescence due to the propidium iodide inside the settled cells indicates that cells are viable after centrifugation.

**Fig. S4** The cell occupancy of each microwell is determined by combined brightfield and fluorescence inspection. Representative micrographs show 10x-objective fluorescence/brightfield images (100 ms exposure time) of the microwell region just prior to scWestern. (Top) Image of a single cell seated in a microwell and (bottom) image of multiple cells seated in a microwell. Using similar micrographs, we exclude scWestern endpoint protein readouts from microwells housing multiple cells.
**Fig. S5** Planar scWestern with passive-gravity settling contains < 4% of microwells filled with single cells and follows the Poisson’s distribution with 100 cells (n = 4 slides for each case, λ = 0.002 for circular, 0.01 for trapezoidal).

\[
U_{drift} = \frac{dx}{dt} = \frac{x \omega^2 (\rho_{cell} - \rho_{liq}) d_{cell}^2}{18 \mu} \\
\int_{r_1}^{r_2} dx = \int_0^{t_1} dt = \int_{r_1}^{r_2} \frac{18 \mu}{x \rho (\rho_{cell} - \rho_{liq}) d_{cell}^2} d_r \\
t_1 = \frac{18 \mu}{\omega^2 (\rho_{cell} - \rho_{liq}) d_{cell}^2} \ln \left( \frac{r_2}{r_1} \right)
\]

**Fig. S6** Time-of-flight for size filtration of SEM cells from U251-GFP cells is calculated by integrating cell drift velocities with respect to radial distance and time. \( U_{drift} \) = cell drift velocity, \( \mu \) = dynamic viscosity, \( \omega \) = rotational velocity, \( r_1 = 0.023 \text{ m}, \) \( r_2 = 0.033 \text{ m}, \) \( \rho_{liq} \) = density of 1x PBS (0.995 g/mL), SEM cell diameter (\( d_{cell} \approx 6 \mu \text{m} \)) and density (\( \rho_{cell} = 1.1 \text{ g/mL} \)), U251-GFP cell diameter (\( d_{cell} \approx 30 \mu \text{m} \)) and density (\( \rho_{cell} = 1.05 \text{ g/mL} \)).
**Fig. S7** scWesterns after sized-based separation of smaller SEM cells from larger U251-GFP cells. SEM only: micrograph from an scWestern of an SEM cell shows a positive peak for the 17kDa H3K79me2 protein, a leukemia-specific protein. Micrograph of a scWestern blot for preferential cell seating of U251-GFP cells into microwells, using size-based separation of SEM cells from larger U251-GFP cells. scWestern reports the presence of U251-GFP cells only, as the H3K79me2 protein peak is absent. Scale bar is 500 μm.

**Fig. S8** GFP, β-TUB, and GAPDH peak intensities from U251-GFP cells are compared between the lab-on-a-disc and the planar (with gravity settling) scWesterns. The lab-on-a-disc and the planar scWesterns have no significant difference in fluorescence-intensity boxplots of GFP, β-TUB, and GAPDH. Blue box ends indicate 25th and 75th percentiles; median value is the red line at box middle; whiskers spread to 95% confidence limits; and red dots indicate outliers. (Mann–Whitney U-test, p-value of GFP = 0.54, p-value of β-TUB = 0.90, p-value of GAPDH = 0.65)
Fig. S9 Measurement of gel thickness for a lab-on-a-disc device indicates an uneven thickness (height) for a dried gel near the microwell array. (A) A surface profile of the dried polyacrylamide gel device reports a thicker gel near the microwell region, as compared to further along the separation axis. (B) A surface profile of the complement SU-8 mold suggests that the dehydration process used prior to antibody probing could be a source of the gel height non-uniformity. Surfaces are profiled using Sloan Dektak 3030.

<table>
<thead>
<tr>
<th></th>
<th>GFP &amp; GAPDH</th>
<th>GFP &amp; β-TUB</th>
<th>GFP &amp; STAT3</th>
<th>GAPDH &amp; β-TUB</th>
<th>GAPDH &amp; STAT3</th>
<th>β-TUB &amp; STAT3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>2.38</td>
<td>3.02</td>
<td>5.68</td>
<td>1.52</td>
<td>5.66</td>
<td>3.67</td>
</tr>
<tr>
<td><strong>STDEV</strong></td>
<td>1.42</td>
<td>1.63</td>
<td>3.33</td>
<td>0.49</td>
<td>3.28</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Table S1 Separation resolutions between each protein peak from the lab-on-a-disc scWestern device.

Notes and references