Integrated microfluidic single-cell immunoblotting chip enables high-throughput isolation, enrichment and direct protein analysis of circulating tumor cells

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Abstract
Effective capture and analysis of a single circulating tumor cell (CTC) is instrumental for early diagnosis and personalized therapy of tumors. However, due to their extremely low abundance and susceptibility to interference from other cells, high-throughput isolation, enrichment, and single-cell-level functional protein analysis of CTCs within one integrated system remains a major challenge. Herein, we present an integrated multifunctional microfluidic system for highly efficient and label-free CTC isolation, CTC enrichment, and single-cell immunoblotting (ieSCI). The ieSCI-chip is a multilayer microfluidic system that combines an inertia force-based cell sorter with a membrane filter for label-free CTC separation and enrichment and a thin layer of a photoactive polyacrylamide gel with microwell arrays at the bottom of the chamber for single-cell immunoblotting. The ieSCI-chip successfully identified a subgroup of apoptosis-negative (Bax-negative) cells, which traditional bulk analysis did not detect, from cisplatin-treated cells. Furthermore, we demonstrated the clinical application of the ieSCI-chip with blood samples from breast cancer patients for personalized CTC epithelial-to-mesenchymal transition (EMT) analysis. The expression level of a tumor cell marker (EpCAM) can be directly determined in isolated CTCs at the single-cell level, and the therapeutic response to anticancer drugs can be simultaneously monitored. Therefore, the ieSCI-chip provides a promising clinical translational tool for clinical drug response monitoring and personalized regimen development.

Introduction
Detection of circulating tumor cells (CTCs) in the peripheral blood has the potential to be a powerful and noninvasive method for the early diagnosis of cancer invasion and metastasis, evaluation of prognosis, assessment of tumor cell sensitivity to anticancer drugs, and monitoring of therapeutic responses. Technologies have been developed based on the distinctive properties of epithelial CTCs and erythrocytes or leukocytes1, such as physical properties (size, weight, or density)2–6, flow, and elasticity characteristics7–10, and differential expression of biological factors11,12. However, simultaneous isolation and downstream molecular characterization of CTCs in the blood of clinical cancer patients remains a major challenge due to the heterogeneity and rarity of CTCs. Compared to simply enumerating and identifying CTCs, evaluating the expression levels of intracellular target proteins in CTCs is more valuable because it provides unique insight into the underlying biological mechanisms of the specific metastasis and neoplastic heterogeneity of tumors. Owing to the low abundance of many intracellular proteins, measuring the changes in protein...
expression in single CTCs requires highly sensitive protein analysis technologies. Recently, many novel single-cell immunoassays and related technologies have been developed for the analysis of proteins, for example, DNA-barcoded antibodies and barcode sequencing\(^\text{13–16}\), metal isotope-labeled antibodies for mass cytometry applications\(^\text{17}\), single-molecule enzyme-linked immunosorbent assays\(^\text{18}\), and plasmonic enzyme-linked immunosorbent assays\(^\text{19}\). However, the use of these methods is restricted to the analysis of free and membrane proteins, and complex operational analysis is often required. Integrated systems for CTC isolation, enrichment, and intracellular functional protein profiling are rare.

Recently, single-cell western blotting has shown great potential for multiplex detection of surface, intracellular, and intranuclear proteins at single-cell resolution. Western blotting is usually regarded as the gold standard for protein analysis because the electrophoretic separation step allows a reduction in antibody cross-reactivity, and the immobilization of an antigen at a physical location on the detection membrane is related to molecular size standards. Single-cell western blotting has enabled the monitoring of intracellular protein expression changes in individual CTCs isolated from patients with primary estrogen receptor-positive (ER\(^+\)) breast cancer\(^\text{20}\). However, micromanipulator and epifluorescence microscopy equipment are required to isolate and transfer the rare cells into microwells, and rare cells are unavoidably mechanically damaged or physically lost during the delay from blood draw to assay.

Herein, we combine the advantages of microfluidic label-free sorting and single-cell immunoblotting techniques to develop an integrated isolation, enrichment, and single-cell immunoblotting (ieSCI) microfluidic system. The ieSCI system permits efficient isolation, high enrichment, and direct molecular functional protein characterization of each rare CTC at the single-cell level (Fig. 1). We demonstrate the use of the ieSCI system for rapid isolation and accurate monitoring of cisplatin-stimulated CTCs to quickly differentiate the rare cell population that is resistant to cisplatin treatment. Furthermore, we demonstrate the clinical application of ieSCI to profile the heterogeneity of single-cell EpCAM expression in patient-derived CTCs.

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**Fig. 1** Schematic illustration of the ieSCI-chip pipeline. a Overview and schematic illustration of the ieSCI-chip operation procedure: (i) zigzag channel-based label-free and high-efficiency cell sorting to isolate CTCs from background white cells in red blood cell-depleted samples; (ii) membrane-assisted CTC purification and enrichment; (iii) gravity-induced settling of CTCs into microwells on the polyacrylamide gel after inversion of the chip; (iv) chemical lysis of CTCs and electrophoretic separation of the released proteins in the customized electrophoresis chamber; (v) in-gel photoimmobilization of the separated proteins by long-wavelength UV irradiation; (vi) immunoblotting with primary antibodies and fluorescence labeling with secondary antibodies. b Timeline of the ieSCI-chip operation procedures.
Results

Design and operation principle of the ieSCI-chip

The operation principle of the ieSCI-chip is presented in Fig. 1a, b and Supplementary Fig. S1 (online). We exploit a zigzag channel as a label-free and high-efficiency cell sorter to isolate CTCs from blood samples. Then, the chip is inverted to feed the isolated CTCs into the microwells for stippling on the hydrogel for single-cell immunoblotting. The hydrogel serves as a molecular sieving matrix for molecular weight-based protein separation through SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and as a protein immobilization scaffold for in-gel immunoblotting. Herein, we employed a MAPmPyTC-modified polyacrylamide gel (referred to hereafter as the MMP gel)\(^{21}\). Previous studies have demonstrated that the photoreactive tetrazole electrophilic addition reaction with a protein as a proximal nucleophile can be completed in a few seconds upon long-wavelength UV irradiation (Fig. 1c)\(^{21}\). Compared with traditional benzophenone-based photosensitive gels, the MMP gel has the advantages of excellent electrophoretic separation ability, high protein photocapture efficiency, and low autofluorescence\(^{21}\). Next, the chip is transferred to a customized electrophoresis chamber, and RIPA-like lysis and a running buffer is then introduced into the chamber. Afterward, the cells are chemically lysed for 10 s, and the lysates of each single cell are subjected to protein separation via electrophoresis. Proteins are immobilized onto the photoactive gel via long-wavelength UV irradiation for 60 s, which is followed by probing with primary antibodies and fluorescent labeling with secondary antibodies. The intensity of the applied electric field is 40 V/cm. The blots are scanned with a confocal microscope to acquire proteomic data. Here, we designed a microchip electrophoresis section on the chip to accomplish in situ single-cell immunoblotting.

The structure of the ieSCI-chip is shown in Fig. 2a. A physical schematic of the ieSCI-chip is shown in Fig. 2b. Figure 2c shows the specific optimized chip dimensions. The competition between the inertial lift force \(F_L\) and Dean drag force \(F_D\) acting on cells inside the zigzag channel gives rise to distinct equilibrium positions for different-sized cells and permits cell separation. In the zigzag portion, cells occupy equilibrium positions in the center of the channel under lower Reynolds number conditions (Supplementary Fig. S2 online). As the Reynolds number increases, large cells (diameter of ~15 \(\mu m\)) distribute near the center of the channel, while small cells flow near the channel walls (Fig. 2d and Supplementary Fig. S3 online). Figure 2e shows the schematic diagram of the particle distribution in the corresponding cross section indicated in Fig. 2d. In the large semicircular channel, when the Reynolds number is low, both the large and small cells keep their equilibrium positions near the...
center of the channel, without a significant separation distance. With increasing Reynolds number, the small cells flow close to the inner wall and large cells flow near the center of the zigzag channel. When the cells flow through the semicircular channel, $F_D$ and $F_C$ become the dominant factors ($a_p \leq 15 \mu m$), which leads the small cells to move toward the inner channel wall, while the large cells ($a_p > 15 \mu m$) move slightly toward the outer wall. When the Reynolds number is higher, the small cells move toward the outer wall, while the large cells move toward the inner wall, making separation difficult.

**Separation efficiency of the zigzag channel portion**

To investigate the flow rate and bifurcation of outlets to achieve higher separation efficiency of CTCs, 10 and 24 $\mu m$ polystyrene particles were used to mimic the hydrodynamic behaviors of WBCs and CTCs, respectively, during the ieSCI-chip optimization process (see Supplementary Materials and Methods). The distances between the 10 $\mu m$ particles and 24 $\mu m$ particles under different flow rates were analyzed with ImageJ©, as shown in Fig. 3a. Under a low Reynolds number condition (Re = 21.1, corresponding to a flow rate of 1 mL/min), the equilibrium positions of the particles were close to each other. When the Reynolds number was increased to 29.6 (corresponding to a flow rate of 1.4 mL/min), the 10 $\mu m$ particles and 24 $\mu m$ particles had the largest separation distance, as shown in Fig. 3b and Supplementary Video S1 (online). When the Reynolds number exceeded 33.9 (corresponding to a flow rate of 1.6 mL/min), the 10 $\mu m$ particles moved toward the outer wall and were difficult to separate. This effect occurred because $F_D$ has a dominant effect over $F_I$ on small particles in the small semicircular channel. The distribution of particles at position 4 (Fig. 2c) at the four-time points under a flow rate of 1.4 mL/min is shown in Fig. 3c. At the four-time points under a flow rate of 1.4 mL/min, the 10 $\mu m$ particles and 24 $\mu m$ particles had the largest separation distance, as shown in Fig. 3c and Supplementary Video S1 (online).

Concentration and purification efficiency of the membrane filter-integrated separation chip

To reduce residual cell background levels after CTC isolation, we further applied a membrane filter-integrated microfluidics sorter to refine the preliminarily separated CTCs. The chip is placed facedown during the separation process to create a superfluous buffer, and the remaining WBCs are filtered through the membrane. Figure 4a, b shows the flow rate simulation results for the ieSCI-chip without a filtering chamber and the standard ieSCI-chip with a filtering chamber obtained with COMSOL© Multiphysics software. The flow rate distribution inside the zigzag channel remained consistent independent of connection to a chamber. The simulation results indicated that the same flow rate of 1.4 mL/min can be adopted to separate CTCs in the ieSCI-chip. To verify the simulation results and evaluate the separation efficiency of the chip with the filter membrane, Dil-stained MCF-7 cells (red) were spiked into DiO-stained WBCs (green) isolated from healthy donors and suspended in PBS solution, and the mixed cell samples were used to investigate the system's ability to separate, purify, and enrich CTCs from blood. The inertial focusing of both the CTCs and WBCs was not influenced by the integration of the membrane filter, and the focusing behavior of cells was consistent with that of cells in the ieSCI-chip without a filtering chamber under a 1.4 mL/min flow rate. Corresponding flow fractions exiting the different outlets were collected and imaged using an inverted fluorescence microscope. Figure 4c
shows the bright field and fluorescence images of the cell compositions in the initial sample, inner outlet, and chamber with or without a membrane filter. The images clearly indicate that the membrane filter improves the purity of CTCs collected in the chamber. ImageJ© was employed to analyze these images, and the purity of CTCs in the chamber is summarized in Fig. 4d. The purity was significantly increased from 68% to 89.92 ± 3.37% with the addition of the membrane sorter.

Furthermore, the whole blood sample needs to be lysed and diluted before processing, and the volume of the blood sample initially injected into the microfluidic chip is 7.5 mL. With the aid of the membrane filter, the final volume of the isolated CTCs should be the volume of the
chamber, which is approximately 0.3 mL. Notably, the CTC enrichment factor can be increased to as high as 25 with the addition of the membrane filter. The high flow rate capability (1.4 mL/min) of the membrane filter-integrated cell sorter allows prompt isolation and enrichment of CTCs within 6 min. Therefore, the developed chip is capable of achieving high-throughput continuous separation and filtration.

**Electrophoresis performance of the integrated ieSCI-chip**

Next, we investigated the influence of the oxygen plasma and drying treatment during chip fabrication on gel electrophoresis performance. To demonstrate whether the photoactive hydrogel after fabrication treatments is compatible for microfluidic electrophoresis and in situ immunoblotting, FITC-labeled bovine serum albumin (BSA, 66 kDa) and ovarian albumin (OVA, 43 kDa for OVA and 86 kDa for dimeric OVA) were employed. The purified proteins were diluted in PBS and used at a final working concentration of 10 μM. The mPyTC-modified PA (MMP) gel was prepared according to a published protocol\(^\text{21}\), treated with oxygen plasma, and dried in an oven for different durations. Purified proteins were added dropwise onto the MMP gel and were then subjected to electrophoresis. Figure 5a shows fluorescence images of the BSA migration behavior in gels subjected to different MMP treatments during the chip fabrication process. The chip fabrication procedures, including both the oxygen plasma and drying treatments, showed negligible effects on the BSA migration distances, with a constant electrophoresis time of 30 s (Fig. 5a, right panel).

We then investigated suitable gel concentrations for electrophoresis on the ieSCI-chip. BSA dilutions were added to the MMP gel in the chamber as previously

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**Fig. 4 CTC separation, purification, and enrichment performance of the ieSCI-chip.**

- **a** Simulation of the flow rate in the zigzag channel without an additional chamber. **b** Simulation of the flow rate in the zigzag channel with an additional chamber connected. **c** Fluorescence images comparing the initial samples and samples collected from each outlet. MCF-7 cells were stained with DiI (red), and WBCs were stained with DiO (green). The scale bar is 100 μm. **d** Comparison of cell purity with or without the addition of the membrane. The data were presented as the mean ± SD values (\(n = 4\)).
To demonstrate the clinical applicability of the method in individualized therapy, we conducted a drug resistance study by using an ER$^+$ breast cancer cell line (MCF-7) to simulate CTCs in ER$^+$ breast cancer patients. The estrogen receptor (ER) is expressed in seventy percent of breast cancer patients. Patients with the same type of ER$^+$ breast cancer, however, show variable clinical responses to routine chemotherapy treatments. Extraordinary cellular diversity and heterogeneity, including EMT, are recognized as critical factors in chemotherapeutic resistance, tumor metastasis, and reoccurrence. Researchers have demonstrated that aberrant alterations in apoptotic pathways in CTCs are potential mechanisms in chemoresistance.

Based on the photoclick cycloaddition reaction illustrated in Fig. 1, the rapid protein photocapture ability of the photoactive polyacrylamide gel facilitates in situ immunoblotting of isolated CTCs on the same chip. MCF-7 cells were treated for 24 h with cisplatin, which is the major chemotherapeutic agent used to treat breast cancer. The half-maximal inhibitory concentration of cisplatin, 5.067 μM, was chosen on the basis of the CCK-8 assay results shown in Supplementary Fig. S6 (online). Then, cells were collected and spiked into WBCs isolated from healthy donors to simulate blood samples obtained from cancer patients receiving chemotherapy. The cancer cells were sorted and enriched in the zigzag channel, and proteins were photoimmobilized on the hydrogel after separation by electrophoresis. Finally, the zigzag channel layer was peeled off, and the remaining section of the
The EpCAM expression level is generally believed to indicate the epithelial-mesenchymal transition status and is related to tumor metastasis and progression. Therefore, evaluation of EpCAM expression in individual CTCs is of clinical significance. We thus employed the ieSCI-chip to confirm the EpCAM expression variations in patient-derived CTCs. The blood sample was incubated with red blood cell (RBC) lysis buffer to deplete RBCs, and all the nucleated cells were resuspended in 4 mL 0.9% saline and injected into the ieSCI-chip. Representative immunoblotting results are presented in Fig. 6c. MCF-7 cells were used as a control and for comparison. Figure 6d displays an overview of the EpCAM expression profiles in MCF-7 cells and individual patient-derived CTCs as measured via the ieSCI-chip system. EpCAM expression exhibited slight uniformity across MCF-7 cells (CV = 17.9%, n = 20) but much more diversity across patient-derived CTCs (CV = 32.8%, n = 11). This might be due to the EMT process, which is a critical process during the metastatic cascade and influences the motility and invasiveness potential of CTCs, or may simply be associated with cellular heterogeneity. However, these differences were not detected when the bulk statistical analysis was used (Fig. 6e). Our results highlighted one major limitation of ignoring or disregarding EpCAM-low or EpCAM-negative CTCs in traditional EpCAM-based CTC detection approaches. The ieSCI-chip is capable of detecting and profiling EpCAM-low/negative CTCs, which may facilitate better prognosis predictions and therapeutic strategy management. In essence, the ieSCI-chip serves as a profiling platform for gauging biological EMT variation among patient-derived CTCs.

### Discussion

CTC characterization is a “liquid biopsy” rather than an Unlike tissue biopsy approach, allowing minimally invasive and real-time biomarker discovery and expression monitoring. However, most of the current clinical applications of CTC detection and monitoring are based on simple enumeration of CTCs. Further characterization, phenotyping, and genotyping of CTCs is needed to...
provide further accurate functional information for evaluating prognosis and guiding personalized treatment. Previous reports have demonstrated the clinical practice of molecular characterization of CTCs through immunomagnetic separation or other methods to investigate the expression of IGFR1, mammaglobins, or urinary plasminogen activator in breast cancer patients and of ERG, the androgen receptor, and PTEN in prostate cancer patients.

In this study, we developed a seamless integrated microfluidic chip for high-throughput, label-free CTC isolation, CTC enrichment, and single-CTC immunoblotting. The proposed ieSCI-chip has the following advantages: (1) highly efficient CTC isolation—the zigzag channel structure increases the length of the separation channel for CTC separation, promoting the capture of rare cancer cells from background blood cells; (2) label-free CTC isolation—CTCs are isolated in an inertial force-based label-free manner, preventing EMT-induced CTC loss or cell stress responses caused by EpCAM-based immunological methods; (3) damage-free CTC purification for direct analysis—the isolated CTCs are conveniently enriched in the presence of a membrane filter without a reduction in cell viability, and purified cells can be directly used for single-cell immunoblotting; and (4) protein profiling at single-cell resolution—the isolated and enriched CTCs are loaded on-chip into an immunoblotting section for protein profiling at the single-cell level, which enables monitoring of protein expression without the need for additional operations.

Of note, this device integrates CTC isolation, CTC enrichment, and single-cell immunoblotting functions in one microfluidic system. By using the integrated ieSCI-chip, we decreased the requirement for costly equipment and significantly reduced the possibility of sample loss during separation and protein analysis. The ieSCI-Chip provides an effective platform to separate CTCs from patient blood samples and to investigate the potential molecular mechanism of chemotherapeutic irresponsiveness and EMT, enhancing the possibilities for
personalized clinical medicine. Dynamic detection of EpCAM expression on CTCs should have prognostic significance in patients with metastatic breast cancer. As a proof-of-concept, we studied EpCAM expression heterogeneity in patient-derived CTCs, and the results were in accordance with previous studies. EMT plays an important role in tumor metastasis and presents a challenge for EpCAM-based CTC isolation and detection methods, such as the CellSearch system. The ieSCI-chip enables efficient CTC capture and characterization without interference from EMT.

Moreover, current mainstream technologies for molecular profiling of CTCs commonly focus on the bulk genome or transcriptome, whereas our chip is designed to characterize protein expression at single-cell resolution. The ieSCI-chip integrates single-cell western blot (scWB) analysis, combining the merits of microfluidics and traditional western blot analysis. Compared with in situ immunofluorescence, whose specificity is limited by antibody cross-reactivity and by limitations on the number of simultaneous detection channels resulting from fluorescence spectrum overlap, scWB can achieve high specificity through SDS-PAGE-based protein separation and high multiplexing ability through multiple rounds of washing and staining. Most notably, we solve the problem of obtaining therapeutic effect predictions by evaluation of biomarkers across individual cells. Additionally, the flexibility in dimension adjustment and flow rate optimization of the zigzag channel facilitates the application of this device not only in breast cancer but also in other diseases, such as lung cancer and cervical cancer (as demonstrated in Supplementary Fig. S1b online).

In this work, we used an inertial focusing zigzag channel consisting of a zigzag portion and a large semicircular channel.

In the zigzag portion, the cells occupy equilibrium positions due to the balance between the inertial lift force ($F_L$) and Dean drag force ($F_D$). These two forces are determined by the channel dimensions, fluid velocity, and cell diameter.

Accordingly, $F_L$ is given as:

$$F_L = \frac{\rho U m^2 a_p^4}{D_h^2} C_L$$  \hspace{1cm} (1)

where $\rho$ is the fluid density, $U_m$ is the maximum fluid velocity defined as $U_m \approx 1.5 \bar{U}$ (where $\bar{U}$ is the average fluid velocity of the fluid in the channel), $a_p$ is the cell diameter, $D_h$ is the hydraulic diameter of the channel defined as $D_h = 2w/(w+h)$ (where $w$ and $h$ are the channel width and height, respectively), and $C_L$ is the lift coefficient.

$F_D$ is caused by the curvature of the channel and is given as:

$$F_D \approx 5.4 \times 10^{-4} \pi \mu D_e^{1.63} \sigma_p$$  \hspace{1cm} (2)

Material and methods
Chip design and fabrication
The separation chip used in our work is a zigzag channel. The widths of the inner and outer outlets are 550 and 950 $\mu$m, respectively. The outer outlet is connected to a 10 mm $\times$ 10 mm chamber. Holes were thoroughly punched in the inlet, outlet, and square chamber (Supplementary Fig. S1b online). The chip consists of five layers. The bottom layer is a microscope slide. A 200 $\mu$m PDMS layer with a 10 mm $\times$ 10 mm chamber on one side was bonded to the glass by oxygen plasma treatment. Then, the 10 mm $\times$ 10 mm chamber was salinized. A PAGE area with microwell arrays was weaved on the 10 mm $\times$ 10 mm chamber. The diameter of the microwells is 60 $\mu$m. The horizontal distance between two adjacent microwells is 400 $\mu$m, and the vertical distance is 2 mm. The glass-bonded PDMS layer with the PAGE area was bonded with the channel layer via oxygen plasma treatment. Before bonding, 30 $\mu$L of water was added to the PAGE area to prevent the gel from drying during the process. In the final step, a filter membrane blocked with 5% BSA was pasted above the square chamber of the chip to prevent nonspecific binding of cells during the filtering process. During the experiment, the ieSCI-chip was inverted on an upright microscope (Eclipse Ci-S, Nikon) equipped with a high-speed CCD camera. The chip was washed with DI water to remove dust, and the square chamber was filled with water before sample injection.

Separation principle of the chip
In this work, we used an inertial focusing zigzag channel consisting of a zigzag portion and a large semicircular channel.

During the experiment, the ieSCI-chip was inverted on an upright microscope (Eclipse Ci-S, Nikon) equipped with a high-speed CCD camera. The chip was washed with DI water to remove dust, and the square chamber was filled with water before sample injection.
where \( \mu \) represents the viscosity coefficient of the fluid and \( D_{c} \) equals the Reynolds number (\( R_e \)) of the channel multiplied by \( \sqrt{D_{r}} \)\(^{41}\).

When cells travel through the large semicircular channel, the equilibrium positions of the cells are determined by the force balance between \( F_{L} \), \( F_{D} \), and the centrifugal force \( (F_{C}) \). According to the literature\(^{42} \), \( F_{C} \) is described as

\[
F_{C} = \frac{\rho_{p} ra^{3} U^{2}}{6r}
\]

where \( \rho_{p} \) is the cell density, \( U \) is the fluid velocity, and \( r \) is the radius of the equilibrium position of the cells in the semicircular channel.

**Single-cell immunoblotting**

Single-cell immunoblotting was performed in accordance with the protocol (Supplementary Fig. S1a online)\(^{43} \). Briefly, after cells were settled into the micro-wells by gravity, they were lysed (~12 s) in the wells in 1× modified RIPA-like electrophoresis buffer prewarmed to 55 °C, and the proteins were then electrophoresed in the photoactive gel at ~40 V/cm (~28 s) in a custom-made electrophoresis chamber. The proteins were immediately photoimmobilized in the gel by a UV-mediated covalent reaction between abstractable hydrogens on the proteins and the mPyTC groups incorporated in the gel matrix (Supplementary Fig. S1a online). During the immunooassay, an antibody in 1× TBST (Tris-buffered saline with Tween 20) with 5% BSA was loaded at the edge of the gel. The gels were probed with primary antibodies for 2 h and with secondary antibodies for 1 h. Between probing steps, the gels were washed two times in 1× TBST (Tris-buffered saline with Tween 20) with 5% BSA.

**Fluorescence imaging and image analysis**

A Zeiss LSM 880 confocal microscope was employed for image acquisition, and ImageJ\(^{50} \) software was used for background subtraction (50 pixels rolling ball radius) and fluorescence quantification of single-cell western blot (scWB) images. Statistical analysis was carried out with GraphPad Prism 8.0.

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**Author contributions**

A.A. and T.Z. contributed equally to this work. A.A., T.Z., H.X., and X.D. conceived the study and experiments; A.A., T.Z., S.L., W.G., and Y.X. performed the experiments, data analysis, and interpretation; A.A., T.Z., A.R.W., N.M., J.L., H.X., and Y.D. wrote the manuscript with input from all authors; and all authors discussed the results and commented on the manuscript.

**Data availability**

Data are available from the authors upon request.

**Conflict of interest**

The authors declare no competing interests.

**Supplementary information**

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