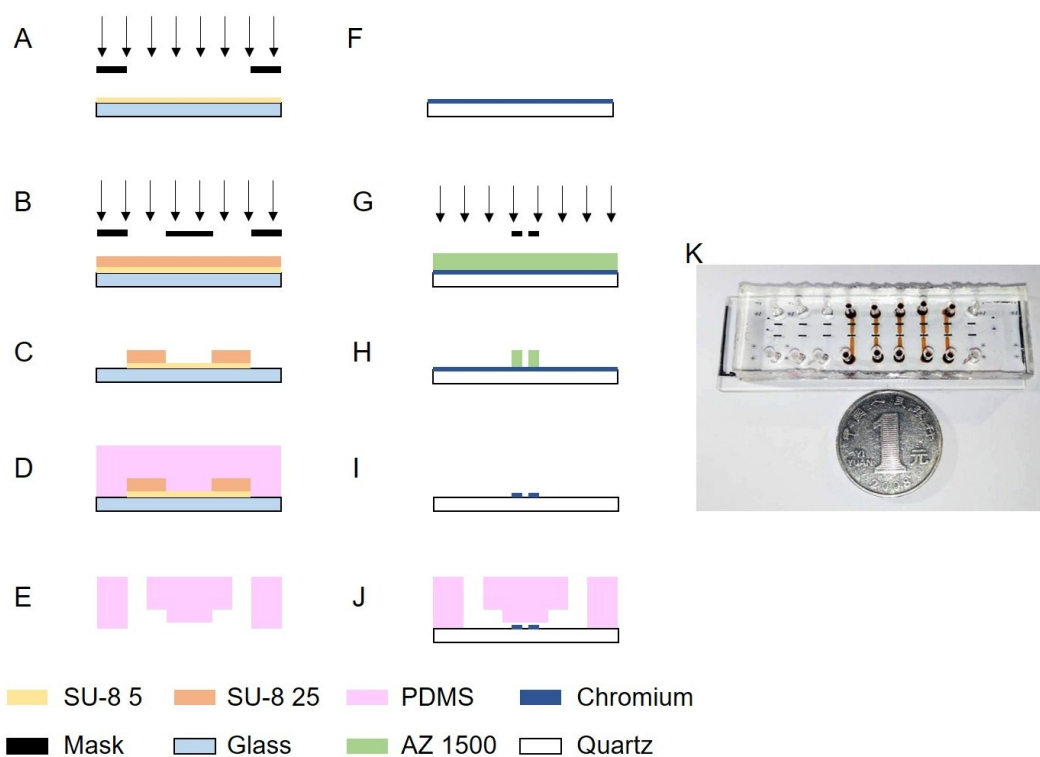
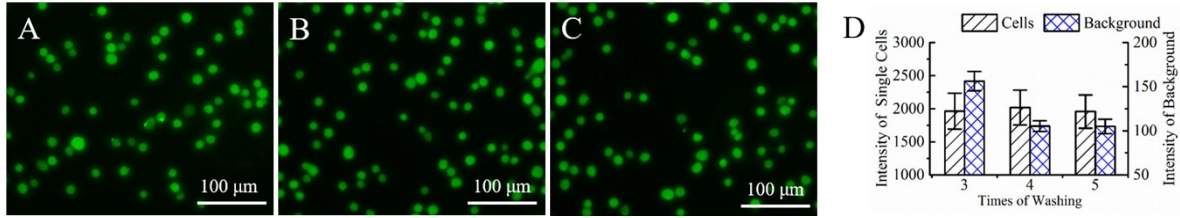


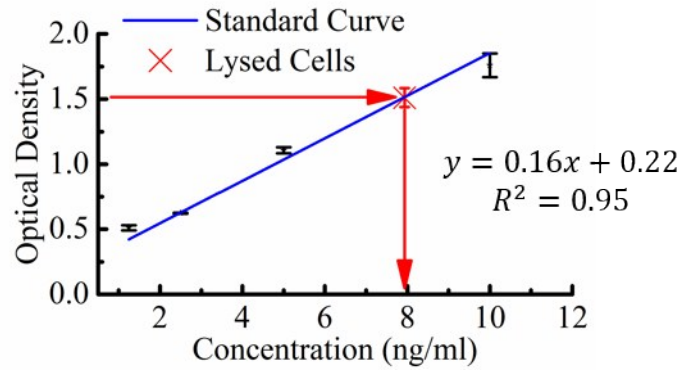
Supplementary Materials



Supplementary Figure 1: Schematic of fabricating microfluidic devices for single-cell protein analysis. The two-layer PDMS device was fabricated based on conventional soft lithography including key steps of (A) SU-8 5 spin coating and exposure without development, (B) SU-8 25 spin coating, exposure with alignment, (C) development, (D) PDMS molding and (E) peeled PDMS with through holes punched. The fabrication of the chromium window was based on conventional hard lithography, including key steps of (F) chromium deposition, (G) photoresist spin coating and exposure, (H) development, and (I) chromium etching. In the end, after plasma treatment, the PDMS layer and the chromium layer were bonded together ((J) and (K)).



Supplementary Figure 2: (A)-(C) Fluorescent pictures of stained single cells after centrifugation and rinsing of 3 times (A), 4 times (B) and 5 times (C), respectively, with quantified fluorescent intensities shown in (D). The fluorescent intensities of single cells in (A)-(C) were quantified as 1963 ± 270 , 2019 ± 263 , and 1957 ± 251 , indicating that further increases in centrifugation and rinsing after three times lead to no further decreases in single-cell fluorescent intensities. Meanwhile, the fluorescent intensities of background solutions in (A)-(C) were quantified as 156 ± 11 , 105 ± 6 , and 105 ± 8 , indicating that further increases in centrifugation and rinsing after four times lead to no further decreases in the fluorescent intensities of background solutions. Thus, four-time centrifugation was used in the following experiments, which effectively removed unbound fluorescence labelled antibodies.



Supplementary Figure 3: The calibration curve obtained by conventional ELISA where a linear correlation between the concentrations of beta actins and optical densities was located. In the experiments, 3.26×10^3 A549 cells were lysed, producing optical densities of 1.51 ± 0.09 , which was translated to $3.57 \pm 0.22 \times 10^6$ copy numbers of beta actins per cell.