Electronic Supplementary Information

Detecting intracellular translocation of native proteins quantitatively at the single cell level

Zhenning Cao^a, Shuo Geng^b, Liwu Li^b and Chang Lu*^{c, a}

^a School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, VA 24061,

USA

^b Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA

^c Department of Chemical Engineering, Virginia Tech, Blacksburg, VA 24061, USA

Email: changlu@vt.edu ; Fax: +01 540-231 5022; Tel: +01 540-231 8681

*Corresponding author

Untreated

 $+TNF\alpha$



Fig. S1 Fluorescence images of untreated cells (A, C) and TNF α -stimulated cells (B, D) after standard immunostaining (A, B) and our selective-release-based immunostaining (C, D). TNF α stimulation was performed by adding 50 ng/ml TNF α for 30 min at 37°C. Selective release was performed by 0.05% saponin for 10 min at room temperature. Scale bar =100µm.



Fig. S2 The optimization of conditions (saponin concentration and treatment duration) for maximal differentiation of untreated and TNF α stimulated cells using selective-release-based immunostaining and flow cytometry. TNF α stimulation was conducted by incubating cell with 50 ng/ml TNF α at 37°C for 30min. Fluorescence histograms were obtained after selective-release-based immunostaining and flow cytometric screening. (A) Various saponin concentrations used when the treatment duration was 10 min. (B) Various treatment durations used while the saponin concentration was 0.05%.