scPICO: Achieving Unprecedented Single-Cell Sensitivity - A Reference-Free Absolute Quantitative Assay of Proteoforms

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Introduction

The single-cell protein interaction coupling (scPICO) assay excels in single-cell proteomics, providing absolute quantification (AQ), protein detection, post-translational modification analysis, and protein-protein interaction insights. It features a streamlined, plate-based workflow without wash steps and boasts an extraordinary signal-to-noise ratio with virtually no background noise. This assay builds upon the prior development of PICO, utilizing a differential partitioning-based readout, two-antibody defined signal and a homogeneous, absolute quantitative assay design [1,2]. The detection unit, referred to as 'couplex' represents the complex formed by the binding of two antibodies with the target protein (see QR code for technology video).

In this study, we utilized the scPICO assay to analyze 4EBP1 protein levels and its phosphorylation (p-4EBP1) in U937 (histiocytic lymphoma) single cells. 4EBP1 plays a crucial role in translation initiation and is a potential target for cancer therapy [3,4]. To showcase the functional capabilities of scPICO, we treated U937 cells with the dual PI3K/mTOR inhibitor, dactolisib [5] and compared scPICO to bulk PICO. The scPICO assay holds promise for advancing single-cell proteomics with its superior sensitivity and capacity for revealing absolute quantitative cellular diversity, paving the way for deeper functional insights into complex biological processes.

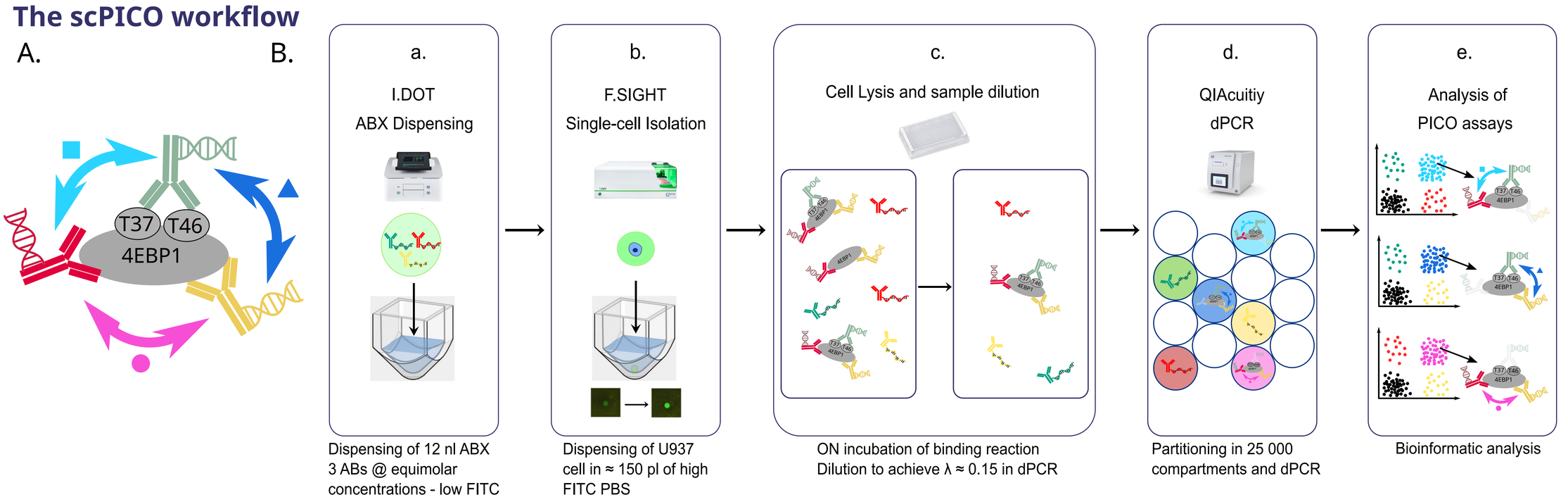

Figure 1. The scPICO workflow. A. Triangular PICO assay, i.e. three assays are set up using pairwise readout of three concurrently binding 4EBP1 (EIF4EBP1 4F3-H2, Invitrogen; 4EBP1 60246-1-Ig, Proteintech) and p-4EBP1 (Phospho-4EBP1 4EB1T37T46-A5, Invitrogen) antibodies at 5.56e-11 M. The assays are indicated with arrows and corresponding colors, while the colors of antibodies match the dPCR fluorescent color channels. The 4EBP1 protein is read using red and yellow antibodies, while phosphorylation of 4EBP1 is measured by two pairs (self-confirmatory assay) red-green and green-yellow, respectively. B. a. Dispensing 12 nL volume of the antibody mix (ABX) in lysis buffer (ACTOME LBTW) under hydrophobic oil (Qiagen Vapor-Lock) in a 384-well plate (eppendorf Microplate 384/V-PP), using the Dispendix I.DOT liquid dispenser, b. and isolating optically interrogated single U937 cells in 150 pL volume and into the 384-well using the Cytena F.SIGHT single-cell dispenser. The previous two steps were fluorescently traced by FITC, to judge successful liquid handling steps. c. After overnight incubation (d) 2% of the material of the single cell was dPCR amplified on the QIAcuity system, and (e) PICO data analysis was carried out to gain the absolute number of detected copies of proteoforms in the dPCR volume.

Results

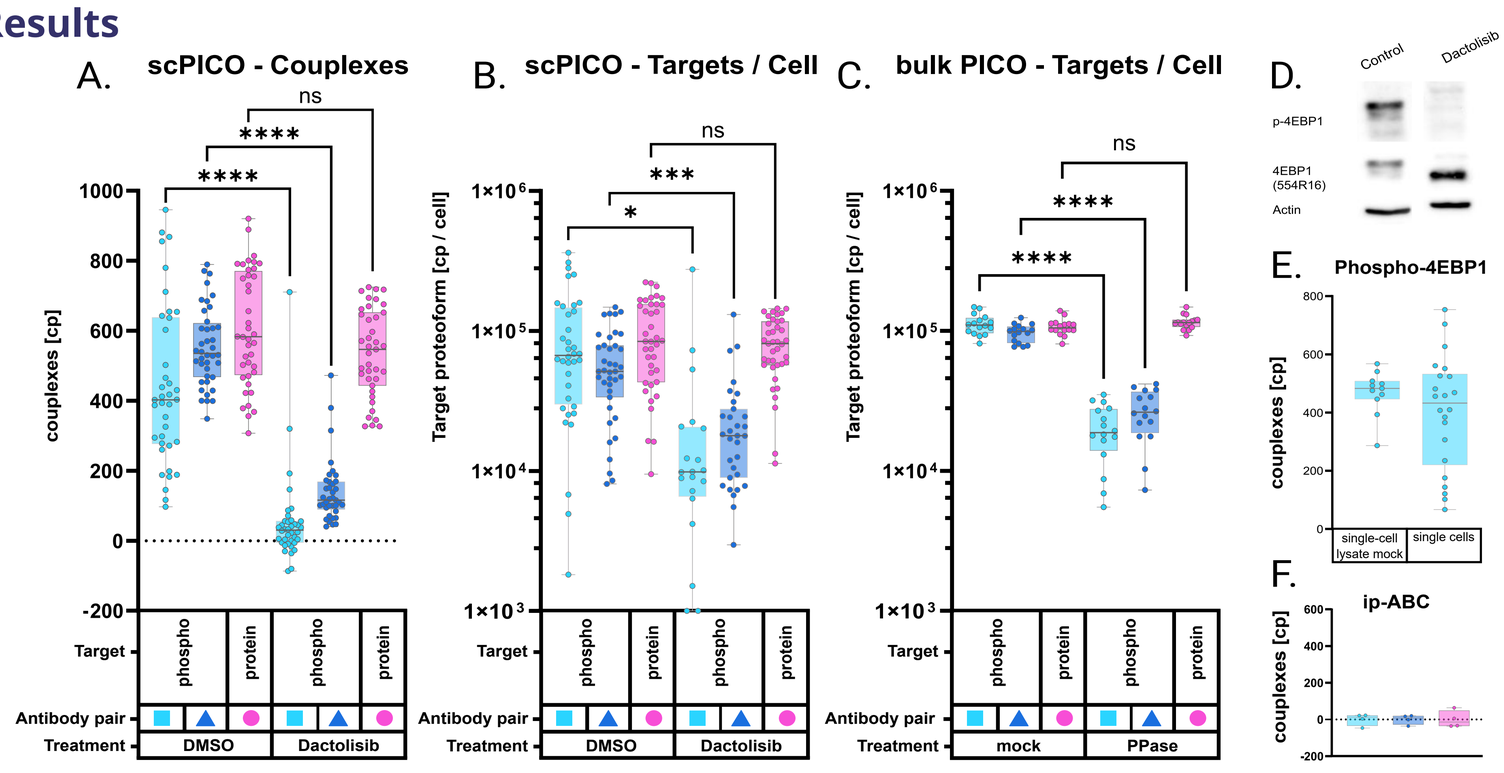

Figure 2: Comparing Bulk and Single-cell PICO. A. scPICO - copies of detected couplexes. scPICO assesses a single cell in a 12 nL volume. U937 cells were treated with dactolisib (5.6 µM) for 4 h or left untreated (DMSO) and subjected to the scPICO workflow. Dactolisib treatment led to a significant reduction in p-4EBP1 signals but not in the 4EBP1 protein signal. Marked heterogeneity in the treatment response was observed among the cell population. B. scPICO - corresponding AQ signal of target proteoforms. C. Bulk PICO assays - 20,000 cells in a 4 uL volume, measuring absolute quantitative amounts (AQ) of 4EBP1 and p-4EBP1 in U937 bulk lysate at 5e-10 M of antibody concentration, treated with λ-phosphatase (PPase) [6] or left untreated (mock). Untreated samples showed comparable 4EBP1 and p-4EBP1 AQ levels, while λ-phosphatase treatment significantly reduced the p-4EBP1 signal, as expected. D. Confirmation of the dactolisib effect on p-4EBP1 by Western blotting. E. Evaluation of the true heterogeneity of scPICO. p-4EBP1 AQ levels in U937 single cells were compared to equivalent amounts of bulk lysate, both analyzed using the scPICO workflow depicting the raw couplex signal (single-cell lysate mock). F. ip-ABC - in-plate negative antibody binding control, using 12 nL volume ABX processed analog to single cells in scPICO but no cell added demonstrating zero signal with no background.

Conclusion

In conclusion, scPICO represents a pioneering reference-free single-cell assay, providing **absolute quantitative (AQ) measurements with exceptional sensitivity down to 2% of the single -cell material**. It accurately quantifies 4EBP1 and p-4EBP1 levels in single cells and **demonstrates zero background noise.** Comparing scPICO to bulk PICO we observed significant heterogeneity in cellular responses. The reliability and **exceptional analytical properties of scPICO was further confirmed** through validation studies, comparing its results to bulk PICO for 4EBP1 protein and Western blot analysis. This breakthrough technology could pave the way for personalized medicine strategies and more effective targeting of diseases, particularly in the context of cancer therapy, where heterogeneity plays a critical role in treatment outcomes.

Contributions

Figure 1 A. and B. Christoph Niemöller and Tobias Groß
Figure 2 A. and B. Christoph Niemöller and Tobias Groß
Figure 2 C. Tobias Hundertmark and Tobias Groß
Figure 2 D. Asmaa Elrakaybi
Figure 2 E. and F. Christoph Niemöller

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