Using geminin as a cell cycle marker to explore single-cell ERK signal dynamics in mutant K-RAS and PI3K MCF10A cells

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To understand signalling dynamics it is crucial to measure the information about the activity of key kinases at the single-cell level. The averaging of protein activity over the entire cell population signals can lead to less accurate results due to cell-to-cell variation. Thanks to state of the art live cell imaging techniques including fluorescent biosensors it is nowadays possible to study the behaviour of single cells in real-time. Here, we present an approach using three fluorescent biosensors to track cells over time (H2B nuclear marker), classify them according to their proliferation and cell cycle state (geminin cell cycle marker) and subsequently extract ERK activity information (ERK-KTR nuclear translocation marker): First, we conducted data exploration of single-cell time series from immortalized human breast epithelial cells (MCF10A) to get an insight into the actual variation between different cells. Then, we created a high-quality dataset containing time series from wild type and two common cancer mutation cell lines (MCF10A-KRAS-G12V, MCF10A-PIK3CA-H1047R) classified by their proliferation status with cell cycle annotation. Finally, we compared ERK signal dynamics between cells in different proliferation and cell cycle states (proliferating, non-proliferating, G0/G1, S/G2/M). With this approach we were able to discover ERK activity differences for the described states and hence providing a proof of concept of how to create a high-quality dataset and study ERK signal dynamics at the single-cell level.

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