Supplementary Information

Digitonin-facilitated delivery of imaging probes enables single-cell analysis of AKT signalling activities in suspension cells

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Fig. S1. Zoom-in images of the U87 cells treated with digitonin. Scale bar: 100 μm.

Fig. S2. Zoom-in images of the THP-1 cells treated with digitonin. Scale bar: 100 μm. Different sized microwells were used in the experiment.

Fig. S3. Zoom-in images of the Jurkat cells treated with digitonin. Scale bar: 100 μm. Different sized microwells were used in the experiment.
**Fig. S4.** The distance matrix generated from the DTW analysis on the THP-1 data set. Each pixel represents the dissimilarity between two corresponding single cells (column and row).

**Fig. S5.** Agglomerative hierarchical clustering results of the distance matrix (THP-1 cells).
Fig. S6. Single-cell AKT signaling trajectories. The clusters are colorcoded (THP-1 cells).
Fig. S7. The distance matrix generated from the DTW analysis on the Jurkat data set. Each pixel represents the dissimilarity between two corresponding single cells (column and row).

Fig. S8. Agglomerative hierarchical clustering results of the distance matrix (Jurkat cells).
Fig. S9. Single-cell AKT signaling trajectories. The clusters are colorcoded (Jurkat cells).