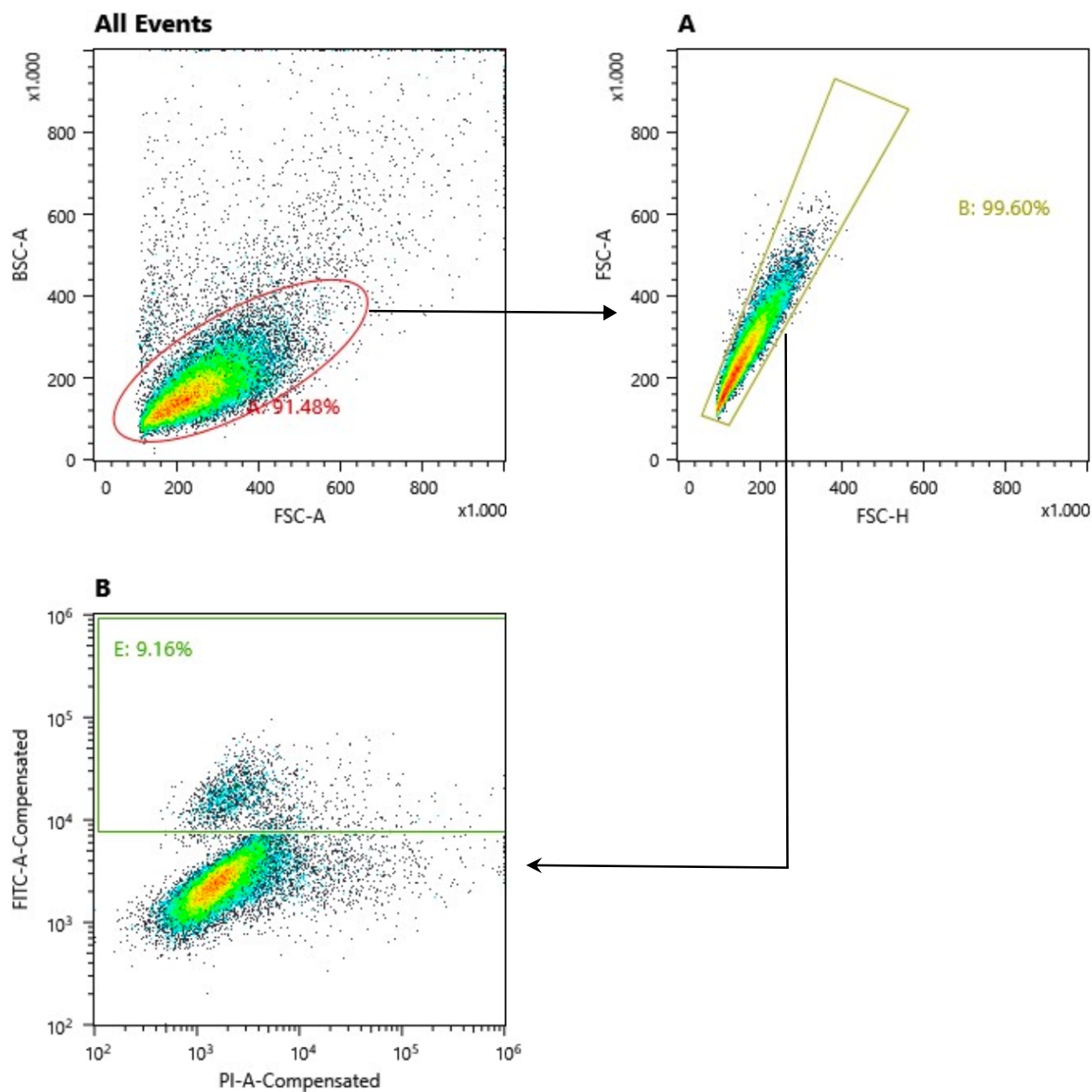


Pooled multicolour tagging for visualizing subcellular protein dynamics

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Supplementary Figure 1. FACS gating strategy. The forward and sideward scatters are used to select the starting cell populations. Single cells are then selected using FSC-A and FSC-H and cells are quantified and/or sorted based on their green or red fluorescence in the FITC and PI channels, respectively. Only the panels with the FITC and PI intensities are shown in the Extended Data Figures of the manuscript. Panel B of the example shown here is part of Extended Data Figure 1d.



Supplementary Figure 2. Additional examples of segmentation. **a-d**, Segmentation for 4 representative fields of view. Tagged channels: overlay of GFP (green), mScarlet (red), BFP (blue). Structural channels: overlay of membrane marker mAmetrine (yellow) and nuclear marker miRFP (red). Scale bars: 50 μ m. **e**, Representative example segmentation of mitotic cells. White arrows point to cells undergoing mitosis, the large majority of which are discarded during filtering. Scale bars: 50 μ m.

