LIVE CELL IMAGING AND QUANTIFICATION OF T CELL RECEPTOR RECYCLING, FROM INTERNALISATION TO RETURN AT THE PLASMA MEMBRANE

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Recycling of plasma membrane receptors after endocytosis is critical to cellular signalling. Sorting of internalised receptors results either in degradation, retrograde transport via late endosomes, or return to the plasma membrane via Rab4- or Rab11-mediated recycling. The mechanisms of cargo sorting into Rab11a recycling compartments are poorly understood. This is partially because following the entire recycling journey of cell surface receptors in live cells is technically challenging. Here we developed a toolkit to visualise and quantify every step of the processes that return internalised receptors to the plasma membrane using TIRF microscopy, and single- and two-photon photoactivation of fluorescent proteins. We show that the T cell receptor (TCR) is internalised into vesicles demarked by the membrane-organising proteins flotillins. We further demonstrate that flotillins are required to sort TCR into a Rab5-Rab11 positive endosomal network seconds after internalisation. This endosomal network eventually mediates a very fast return of TCR to the cell surface in Rab11-positive vesicles. Combining a) the visualisation of recycling from intracellular compartment by two-photon photoactivation of fluorescent proteins and b) optogenetic aggregation of endosomes, we further confirm that Rab5- and Rab11-positive endosomes function together to return internalise TCR to the plasma membrane. Finally, we show that the spatial organisation of the TCR-, Rab5- and Rab11-positive endosomes is altered in flotillins knock-out cells. Together, our data suggest that flotillins support the recycling of TCR by acting as an entry door at the plasma membrane to a Rab5-Rab11 positive endosomal network and by maintaining the architecture of these endosomes.