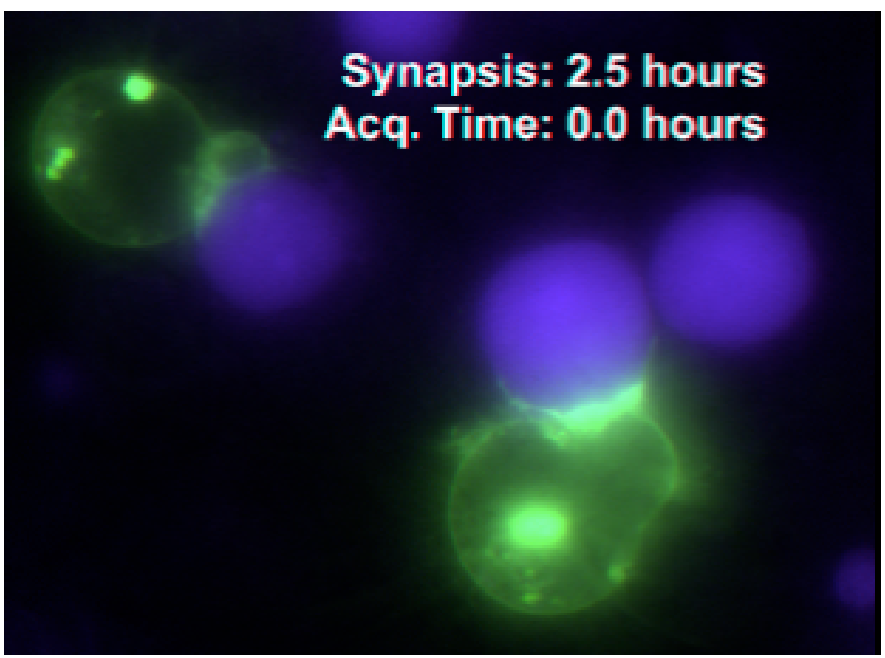
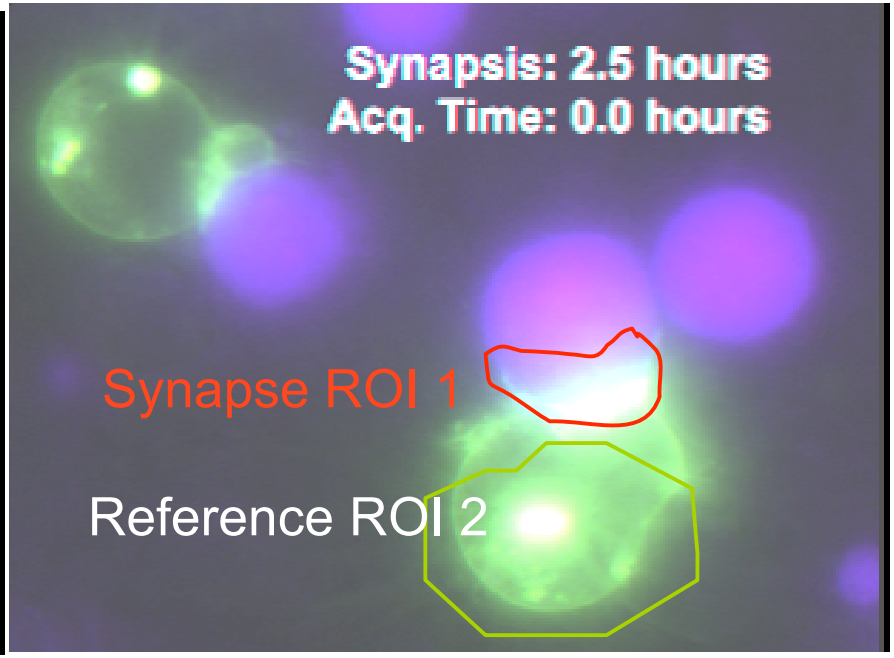
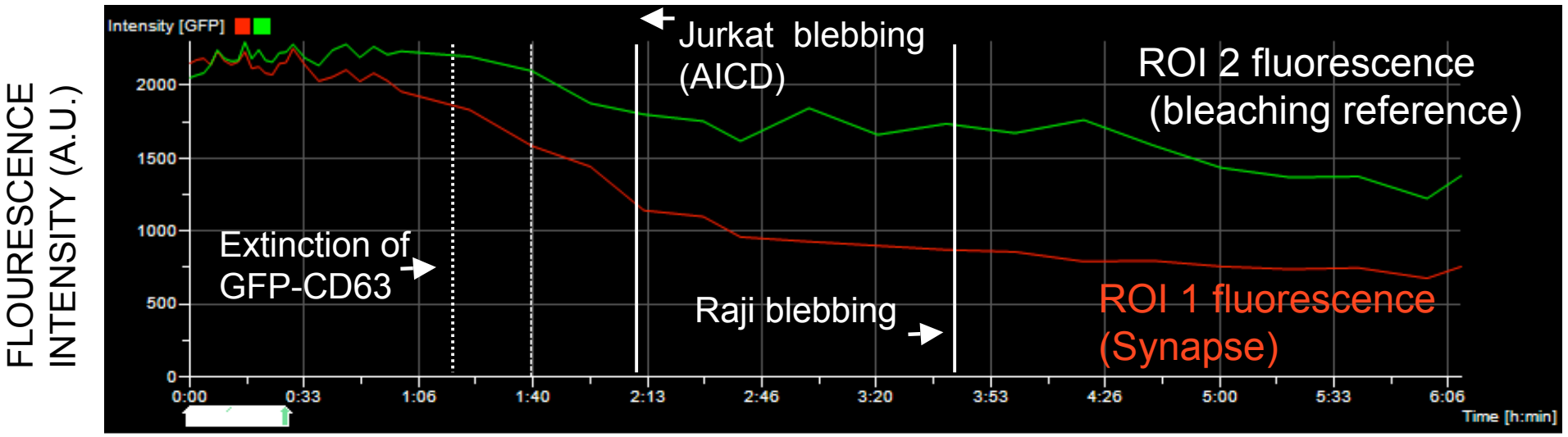
The extinction of MVBs at the synapse occurs before the initiation of AICD







Time elapsed after synapse formation (h:min)

Video-lapse analysis for the Referee #1.

The extinction of MVBs at the synapse area occurs before AICD.

Raji B cells labelled with cell tracker blue (CMAC, blue) were pulsed with SEE for 30 min and synapses with Jurkat cells expressing GFP-CD63 (green) were formed as indicated in Material and Methods. 2.5 hours after synapse formation, simultaneous adquisition of GFP-CD63 and CMAC fluorescence was performed and the fluorescence analysis carried out using NIS-AR software. In the upper panels, the first frames corresponding to a representative "raw" video (left side) and the same, "overexposed" video (right side) for the Referee are represented. The "overexposed" video is included to favour the visualization of the plasma membrane blebbing. The average fluorescence intensity of GFP-CD63 corresponding to two different regions of interest (ROI 1 and 2) in the "raw" video were measured and plotted versus the time elapsed after synapse formation (lower panel). The ROI 1 (red color) corresponds to the synapse area, whereas ROI 2 (green color) was taken as a bleaching reference for the GFP-CD63 fluorescence. As seen in the fluorescence diagram, the decrease in the GFP-CD63 fluorescence at the synapse ROI (red line) started around 1:20 (h:min) after synapse formation, and the first sign of AICD of the Jurkat cell (plasma membrane blebbing) was detected after 2:30 (h:min). A representative example out of 20 synapses recorded is shown.