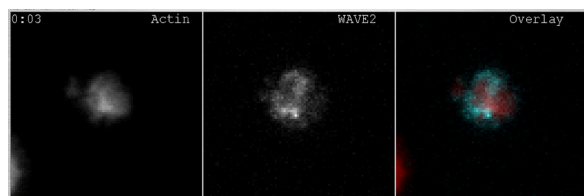
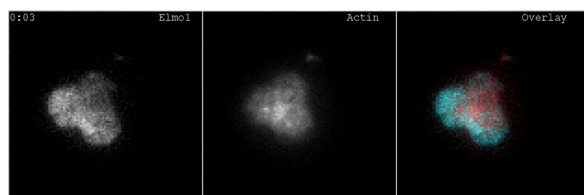


## SUPPLEMENTAL MATERIAL

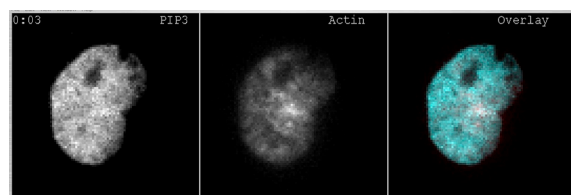
Le Floc'h et al., <http://www.jem.org/cgi/content/full/jem.20131324/DC1>



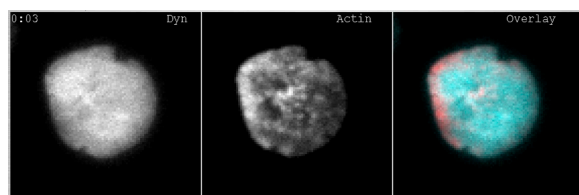
**Video 1. F-actin and WAVE2 localization at the IS.** OT-1 CTLs expressing RFP-labeled Lifeact together with GFP-labeled WAVE2 were analyzed by TIRF microscopy (60× magnification) on lipid bilayers containing pMHC and ICAM-1. A 15× time-lapse video is shown, with time displayed (minutes:seconds) in the top left corner. Bars, 5  $\mu$ m. The video is representative of at least 160 cells imaged over four independent experiments.



**Video 2. F-actin and Elmo1 localization at the IS.** OT-1 CTLs expressing RFP-labeled Lifeact together with GFP-labeled Elmo1 were analyzed by TIRF microscopy (60× magnification) on lipid bilayers containing pMHC and ICAM-1. A 15× time-lapse video is shown, with time displayed (minutes:seconds) in the top left corner. Bars, 5  $\mu$ m. The video is representative of at least 120 cells imaged over six independent experiments.



**Video 3. F-actin and PIP<sub>3</sub> localization at the IS.** OT-1 CTLs expressing RFP-labeled Lifeact together with GFP-labeled Grp1PH were analyzed by TIRF microscopy (60× magnification) on lipid bilayers containing pMHC and ICAM-1. A 15× time-lapse video is shown, with time displayed (minutes:seconds) in the top left corner. Bars, 5  $\mu$ m. The video is representative of at least 80 cells imaged over four independent experiments.



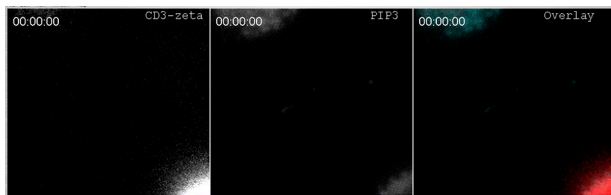
**Video 4. F-actin and Dyn1 PH domain localization at the IS.** OT-1 CTLs expressing RFP-labeled Lifeact together with GFP-labeled Dyn1PH were analyzed by TIRF microscopy (60× magnification) on lipid bilayers containing pMHC and ICAM-1. A 15× time-lapse video is shown, with time displayed (minutes:seconds) in the top left corner. Bars, 5  $\mu$ m. The video is representative of at least 80 cells imaged over four independent experiments.



**Video 5. Localization of the p85 $\alpha$  subunit of PI3K at the IS.** OT-1 CTLs expressing GFP-labeled p85 $\alpha$  were analyzed by TIRF microscopy (150 $\times$  magnification) on lipid bilayers containing pMHC and ICAM-1. A 30 $\times$  time-lapse video is shown, with time displayed (minutes:seconds) in the top left corner. Bar, 5  $\mu$ m. The video is representative of 25 cells imaged over two independent experiments.



**Video 6. Localization of the p85 $\beta$  subunit of PI3K at the IS.** OT-1 CTLs expressing GFP-labeled p85 $\beta$  were analyzed by TIRF microscopy (150 $\times$  magnification) on lipid bilayers containing pMHC and ICAM-1. A 30 $\times$  time-lapse video is shown, with time displayed (minutes:seconds) in the top left corner. Bar, 5  $\mu$ m. The video is representative of 40 cells imaged over two independent experiments.



**Video 7. Annular PIP<sub>3</sub> patterning coincides with the formation of a central cluster of TCR.** OT-1 CTLs expressing RFP-labeled CD3 $\zeta$  together with GFP-labeled Grp1PH were analyzed by TIRF microscopy (150 $\times$  magnification) on lipid bilayers containing pMHC and ICAM-1. A 15 $\times$  time-lapse video is shown, with time displayed (minutes:seconds) in the top left corner of each image. Bars, 5  $\mu$ m. The video is representative of 17 cells imaged over two independent experiments.