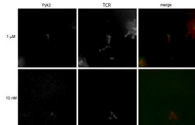
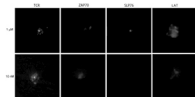


## SUPPLEMENTAL MATERIAL

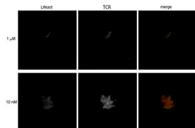
Hashimoto-Tane et al., <http://www.jem.org/cgi/content/full/jem.20151088/DC1>



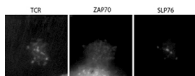
**Video 1. TCR-MCs transiently bordered by Pyk2 in the initial phase of activation.** TIRF videos of TCR (H57)-Dy549 and Pyk2-GFP in AND-tg T cells placed on 1  $\mu$ M or 10 nM Ag-loaded planer bilayer were taken at the rate of one frame per 5 s for 3 min. In the merged images, green and red show Pyk2-GFP and TCR, respectively. The fields of 18- $\mu$ m squares for each color or merged videos are arranged.



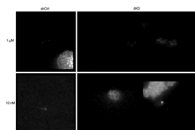
**Video 2. Different behaviors of ZAP70 and SLP76 clusters upon weak stimulation.** TIRF videos of TCR (H57)-Dy549, ZAP70-GFP, SLP76-GFP, and LAT-GFP in T cells placed on 1  $\mu$ M or 10 nM Ag-loaded planer bilayer were taken at the rate of one frame per 5 s for 6 min. The fields of 18- $\mu$ m squares for each color or merged videos are arranged.



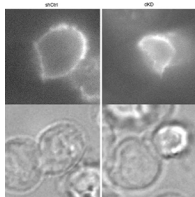
**Video 3. F-actin transiently accumulated at TCR-MCs in the initial phase of activation.** TIRF videos of TCR (H57)-Dy549 and Lifact-GFP in T cells placed on 1  $\mu$ M Ag-loaded planer bilayer were taken at the rate of one frame per 5 s for 5 min. In the merged images, green and red show Lifact-GFP and TCRs, respectively. The fields of 18- $\mu$ m squares for each color or merged videos are arranged.



**Video 4. Disappearance of SLP76 clusters and remaining TCR-MCs and ZAP70 clusters by CytD treatment.** TIRF videos of TCR (H57)-Dy549, ZAP70-GFP, and SLP76-GFP in T cells placed on the planar bilayer containing 1  $\mu$ M Ag taken at the rate of one frame per 5 s for 5 min. At the 11th frame, CytD was added at a final concentration of 10  $\mu$ M. The fields of 14.5- $\mu$ m squares are arranged.



**Video 5. The unstable contacts and impaired IS formation of dKD cells on the planer bilayer.** TIRF videos of TCR (H57)-Dy549 in shCtrl and dKD cells on 1  $\mu$ M or 10 nM Ag-loaded planer bilayer were taken at the rate of one frame per 5 s for 5 min. The fields of 22- $\mu$ m squares are arranged.



**Video 6. The unstable contacts and impaired IS formation of dKD cells upon interaction with APCs.** The videos of TCR (H57)-Dy549 and a differential interference contrast image in shCtrl and dKD cells with 1  $\mu$ M Ag-loaded activated B cells were taken at the rate of one frame per 10 s for 8.5 min. The fields of 14- $\mu$ m squares are arranged.