## SUPPLEMENTARY MATERIALS & METHODS

### Fluorescence resonance energy transfer (FRET) microscopy

Cells were plated at 1-2x$10^5$/$cm^2$ on poly-L-lysine-coated glass coverslips and allowed to bind for 7 min at room temperature, then labeled on ice for 30 min with anti-TCRβ (1/40 IP-26, mouse IgG1; eBioscience) and with anti-CD8α (1/40 UCHT-4, mouse IgG2a; Sigma-Aldrich) or anti-CD4 (1/40 RPAT-T4, mouse IgG1; eBiosience) diluted in PBS/0.2% BSA (Sigma-Aldrich). After three washes in the same cold buffer, cells were fixed at room temperature for 20 min with 4% formaldehyde and 0.1% glutaraldehyde (Sigma-Aldrich) in 0.1 M phosphate, washed twice in PBS/BSA and incubated with 10 mM glycine in PBS for 10 min, then incubated for 30 min on ice with anti-IgG2a-Alexa 488 antibody (1/400, donor, green; Invitrogen), and anti-IgG1-Alexa 568 secondary antibodies (1/400, acceptor, red; Invitrogen) diluted in PBS/BSA. After three washes, cells were fixed again and coverslips were mounted onto glass slides using Prolong Gold (Invitrogen). Images were acquired with an LSM 510 laser scanning microscope and analyzed by AIM Software (Zeiss). Imaging was performed with a 488 nm line generated by an Ar laser (30 mW) used at 1% and a 561 nm line generated by a DPSS laser (10 mw) used at 1%. Acceptor photobleaching was achieved using the DPSS laser at 100% with 100 iterations. Three images were recorded before bleaching and after bleaching. To calculate the increase in donor emission (indicative of FRET efficiency), for each cell, three regions of interest were chosen, bleached, and compared with three control regions in not bleached area. Increase in donor emission was calculated as follows: % FRET efficiency = [1 – (donor intensity before bleaching / donor intensity after bleaching)] x 100. Mann-Whitney U test was performed with PASW Statistics 18.0.0 (SPSS Inc.).